

Production rules down on the farm

USDA's "payment-in-kind" programme has had a stunning — and expensive — success in getting farmers to grow less. Good research would have been a better investment.

EIGHTY-TWO million acres of corn (maize), wheat, sorghum, rice, and cotton land are lying idle in the United States this year as a result of the most successful — and most expensive — acreage diversion programme attempted by the US Department of Agriculture (USDA) since the inception of such programmes during the New Deal. Preliminary estimates of the cost range from \$20,000 million upwards. And while the programme is by its own criteria a success — production dramatically reduced, commodity prices increased (December corn futures are now more than \$3 per bushel, up from less than \$2 last year) — it is hardly the sort of solution that either politics or economics will allow to be lasting. The pity is that so little funding is being channelled to fundamental solutions of the farmer's perennial plight of overproduction, low prices and unstable markets.

Agricultural research has deeper roots in the federal government than do any of the farm support programmes, and even the most general of policy objectives seems to leave research untouched. The land-grant colleges and state experimental stations, established a century ago, still form the backbone of US agricultural research. These institutions, traditionally focusing on local needs, have never really been subject to a coherent national policy. Their funding is set by a state-by-state allocation formula dating back to the days of their founding, and no national goals — or even scientific peer review — can alter that pattern of funding.

The diversity of the system, to be sure, together with its responsiveness to local problems, accounts for much of American agriculture's phenomenal success. Yet this was truer in the past than it is today. Rapid communication has eliminated some of the justification for 50 separate research institutions spread across the country. At the same time, the benefits to be gained from management improvements and technological application, which are of a fundamentally regional character, are shrinking compared with the potential benefits of a fundamental expansion of our understanding of plant genetics, to choose one obvious example.

The state-by-state system of agricultural research seems an anachronism, especially when contrasted with the way the National Institutes of Health or the National Science Foundation operate. Even worse than the fact that agricultural research funds are doled out without regard to the scientific merits of the projects is the exclusion of non-land-grant universities from the system. Harvard, Stanford and Massachusetts Institute of Technology, to name a few universities that might contribute much to plant genetics, are not eligible to receive USDA research funds. The best molecular genetics students at these institutions have no good reason to choose plant sciences over medical sciences when they see where scholarships and traineeships are available; young researchers face a similar foregone choice.

A small programme of competitive, peer-reviewed grants has been run by USDA for five years, but it represents a drop in the ocean; \$17 million, compared with the \$244 million federal contribution to the land-grants. One is tempted to push the comparison further and cast an eye at the \$20,000 million to be spent this year on commodity price support. The comparison is in fact not unfair. Like all farm programmes since the New Deal, this year's "payment-in-kind" programme seeks to support commodity prices in exchange for (and, in part, by way of) decreased production. Farmers face increasing costs and inadequate prices;

as "price-takers", they not infrequently face a market that will not pay them for the costs of production.

The lynchpin of the price support programmes for field crops is the Commodity Credit Corporation (CCC), one of the most successful and enduring of the New Deal initiatives. CCC offers farmers a loan for their crops; the farmers, in turn, pledge the crop as collateral. If the market price exceeds the loan price, the farmer sells his crop and repays the loan. If the market price drops below the loan price, he can choose to default on the loan, in effect selling the government his crop. In accepting the loan, the farmer agrees to set aside some percentage of his acreage.

CCC's stockpiles of commodities have been growing in recent years, and in a move to unload its stocks and, according to plan at least, save the government some money, farmers were offered a different deal this year: in exchange for not planting large numbers of acres, they would receive a government payment in the commodity they would have planted. This payment-in-kind scheme went over so well that USDA is now encouraging farmers to grow wheat and voluntarily default on their CCC loans so that USDA will have enough wheat with which to pay farmers. USDA has requested \$325 million to buy this wheat which it needs to pay farmers not to grow wheat.

The emphasis in all of these support programmes is on the production side. Similarly, to the extent that goals in agricultural research are articulated, they too emphasize production. Missing is any consideration of the costs farmers face — and what really matters to farmers is not production, not even price *per se*, but profit margin. Rather than spend thousands of millions of dollars supporting prices, a modest investment might be contemplated in reducing costs. Plant genetics would not be a bad place to start. The possibility of engineering as much technology as possible into the genes of the seed is surely the most direct route to reduced input costs. And more efficient crop varieties and resistant varieties could make dramatic cuts in fertilizer and pesticide expenses.

Surely some of the \$20,000 million being poured into price supports can be more wisely invested in pursuit of long-term dividends. The \$17 million allocated for competitive grants in agriculture is an embarrassingly small investment in good science — especially when good science may hold the answer to the perennial public policy problem of keeping farms in business. □

Thatcher's man

The scientific adviser to the British Prime Minister may be too close for comfort.

THE shape, if not the plans, of Mrs Thatcher's new government is now clear. Armed with a greatly increased majority from a slightly decreased proportion of the total votes cast, she has quickly disposed of those members of her past government who had publically argued for moderation along with those who had not noticeably argued for anything. One victim of the latter category is Mr William Shelton, who returns to the backbenches to make way for fewer, newer (see page 650) junior ministers to assist the unpopular, but retained, Sir Keith Joseph in the Department of Education and Science. Also retained, amongst other things to denationalize British Telecom, is Mr Kenneth Baker, Minister for Industry and Information Technology within the newly



combined Department of Trade and Industry.

A third survivor of interest to the science community is Mr Robin Nicholson, not a member of government or even parliament. Mr Nicholson, erstwhile professor of metallurgy at the University of Manchester and managing director of Inco Europe, has been chief scientist on the Central Policy Review Staff since 1981. Last week Mrs Thatcher disbanded the Central Policy Review Staff but retained Mr Nicholson as chief scientific adviser to the Cabinet Office. The question is whether his role will be strengthened by the shift in circumstances.

The Central Policy Review Staff, commonly known as the Think Tank, was established by then-Prime Minister Edward Heath in 1971. Its handful of bright young thinkers, at first under Lord Rothschild, were meant to provide the Cabinet with advice upon any issue where there was a need either to counter the prevailing advice of ministries or to think a good deal further ahead than most members of a Cabinet can manage to.

Widely credited both with some very successful forecasting, such as that of the energy crisis in 1973, as well as some foolish predictions, the Think Tank was unfortunately bound to keep most of its advice confidential. The laundered versions of some of its views on scientific and technological matters can be found in a series of booklets produced by the Advisory Committee on Applied Research and Development.

The leaked versions of some of its more recent views on a variety of matters are one cause of its demise. More importantly, the Central Policy Review Staff were beginning to appear redundant to Mrs Thatcher's needs as she built up her own bevy of personal advisors. Those have now been supplemented with a few of the more useful members of the Central Policy Review Staff, Mr Nicholson among them. Its other members will neither perceptibly nor, one imagines, for long, swell the ranks of the unemployed.

As a consequence of his new circumstances, Mr Nicholson will be much more his own man than before but not necessarily the better for it. For one thing he will no longer have access to the constructive criticism that was automatically available from other members of the Central Policy Review Staff, each with their own expertise. Nor will he find it so easy to contribute his advice on topics that are not, by definition, primarily concerned with science. The danger for Mr Nicholson and those who may follow him is that of becoming no more than a tame (or not so tame) advisor on those few subjects that are clearly labelled science and technology without ever extending into less obvious areas where it is nonetheless important that scientific aspects are taken into account. His success in reaching far and wide will now depend to a considerable extent upon his personal relationship with Thatcher. A metallurgist may be just the person to relate well to a lady of iron, but a system that depends so heavily on such vagaries is not a healthy one.

Nuclear power play

The time is not ripe to give the American nuclear industry free reign.

THE Reagan Administration will have only itself to blame if its latest effort to resuscitate the moribund nuclear power industry is thwarted by a cautious Congress. There is considerable sympathy in the Senate — and somewhat less in the House — for the administration's belief that the labyrinthine licensing procedures of the Nuclear Regulatory Commission (NRC) are a significant cause of the industry's woes. There are 83 licensed nuclear power plants in the United States and their number will grow to more than 100 within the next ten years. But the industry has been wracked by cancellations and it is five years since anyone has placed an order for a new plant. That that part of the industry's predicament can be blamed on the regulators is beyond dispute. But by trying to cut too clean a swathe through NRC's procedures (see page 650) the administration is likely to succeed only in panicking the public, on whose support the fate of nuclear power ultimately depends.

It has always been a peculiarity of the licensing procedure that utilities cannot obtain operating permits until the plants they have built are substantially complete. Utilities have every right to assume that they should be allowed to turn on enormously expensive facilities whose construction has already been sanctioned by NRC. In practice, and notwithstanding the current impasse at the Shoreham plant on Long Island, it is unlikely that any plant will be allowed to stand idle permanently. Its controversial decision this month to retract a threat to close the reactors at Indian Point is ample evidence of NRC's sensitivity to the economic dangers of too inflexible an adherence to safety regulations. But there is some justice in the industry's complaint that the eleventh hour application for an operating licence, and the public hearings that ensue, introduce needless delays and uncertainties.

The administration wants to solve this problem by merging the application for a building permit and an operating licence into a single stage. The appeal of the suggestion is that it would compel utilities to present NRC with much more complete design plans early in the building process, while removing the fear of investing in a plant which might never be allowed to operate. The drawback is that the industry's uneven safety and construction record means NRC will still eventually have to ensure that the plant had been constructed safely in accordance with the approved design. For this reason, the administration proposal that the utility itself be empowered to certify that the work has been completed satisfactorily cannot be accepted. Nevertheless, there is every reason to commend the alternative legislation, drafted by the commission, which would create a single-stage licensing procedure subject to a final pre-operation inspection by NRC.

It will be objected that a combined building/operating licence is hardly worth the paper it is written on if it can be overturned after a subsequent review by NRC. But the new procedure will be no more ambiguous than the existing arrangements, which hold out to public intervenor groups the false hope that last-minute objections at the pre-operation stage offer an opportunity to doom a completed reactor. What the change will do is transfer the burden of proof, so that a utility that pays meticulous attention to meeting NRC specifications will have little to fear from the final review.

The merits of ending two-step licensing are, however, certain to be overshadowed by the demerits of other elements in the draft legislation. Perhaps most objectionable is a proposal to constrain the authority of NRC to impose design modifications on a plant once it had granted a building licence. Complaints by the industry that capricious "backfitting" by NRC is responsible for many of the extra costs incurred by nuclear power plants are only partially true. At least as much blame can be laid at the door of contractors who design as they go and fail to ensure that plants are properly built. It is inconceivable that public opinion will accept rigid constraints on NRC's authority to order design modifications at the same time as moving towards a one-step licensing procedure. For all the talk of the new maturity of the industry 29 years after NRC began to license the construction of power plants, nuclear power remains an evolving technology of which there is enormous public suspicion.

There is, in the administration's proposals, a disturbing confusion of ends and means. The administration rightly believes that the industry could become highly economical if it moved seriously towards the standardization of plant designs and enabled NRC to pre-approve generic designs. But it exaggerates the ability of the regulatory process to provide incentives for such moves to take place. NRC reflects the industry more than it shapes it. The fact that some NRC procedures are cumbersome and occasionally makeshift is not unconnected to the fact that the industry itself is highly fragmented, with some 60 companies either running or building nuclear plants and thereby creating a bewildering variety of equipment and procedures. Superimposing a deceptively neat regulatory process will do little to change the structure of the industry and is likely to increase public suspicions. There is a growing consensus that some licensing reform is overdue, but nobody is yet ready to provide the builders of nuclear power plants with the regulatory equivalent of a blank cheque.

Chemical weaponry

Thumbs-down for binary weapons from US Congress

THE Reagan Administration's military spending programme was dealt a blow last week when the House of Representatives, by 216 to 202, refused to fund production of new chemical weapons. This is the second year running that the administration has lost in its bid to go ahead with production of new "binary" chemical shells and bombs, which it claims are needed to replace deteriorating stocks of older chemical munitions and to maintain a deterrent against the Soviet Union. The United States has produced no chemical weapons since 1969, when then-President Nixon ordered a unilateral halt to production. Unlike the proposed binary weapons, which contain two relatively benign chemicals that are mixed to produce the lethal agent, the older weapons contain live nerve agent.

The vote to delete the \$115 million requested to begin production came amid disclosures of serious design flaws in one of the proposed weapons, the "Big Eye" bomb. Last month, the US Army conceded that the bombs could explode prematurely, and dropped \$43 million from the production request. The remaining \$115 million would have gone towards production of 155-mm shells but would also apparently have been used to build production lines for the Big Eye and even to begin manufacture of the bomb casings.

According to Representative Ed Bethune (Republican, Arkansas), who sponsored the amendment to delete production funding along with Representative Clement Zablocki (Democrat, Wisconsin), Big Eye had been designed for a high altitude drop. As originally conceived, the two chemicals in the bomb would be mixed before release. Bethune said that the army realized only recently that a high-altitude attack was out of the question because the aircraft would be detected by radar, and when they began testing at low altitudes, they discovered that the high temperatures could cause sufficient gas pressure within the bomb to rupture it.

The administration engaged in a heavy lobbying effort in support of its request for funds. Proponents, backed by letters from the Department of Defense and from Secretary of State George Shultz, argued that progress on a treaty banning possession of chemical weapons could only be achieved by demonstrating US resolve to build the new weapons. The lobbying effort even included the screening of a film, produced by the army only two weeks ago, of a successful test of Big Eye. During debate on the amendment, Bethune heatedly countered that the test involved dropping the bomb from 6,000 feet and

starting the mixture only after the bomb was dropped. Bethune said that 15 to 20 seconds are needed for the reaction to run to completion, making this approach totally impractical for low-altitude attacks. And he said "they would be shot down in a minute" flying at 6,000 feet if it were a real mission.

The House action did not affect the approximately \$900 million that the administration had requested for chemical defence and research on chemical weapons. Nor did it appear to be indicative of general opposition to the Reagan defence programme. By wide margins, the

House rejected amendments seeking to delete other weapons systems. A request for \$19 million with which to begin deploying antisatellite weapons (ASATs) was approved despite warnings from Representative George Brown (Democrat, California) that the eventual cost would reach tens of thousands of millions and would all but rule out the possibility of a verifiable ban on ASATs. The US Air Force is planning a test this summer of ASAT, which is a nonexplosive projectile launched from an F-15 fighter. Research and development costs of the project have already reached \$1,000 million. Funds for the B-1 bomber and for the army's controversial computer-controlled anti-aircraft gun, known as DIVAD or the "Sergeant York", were likewise approved. The contentious question of funds for deployment of the MX missile will be taken up after the House returns from its 4 July recess.

Stephen Budiansky

Spina bifida

MRC folate trials to start at last

THE Medical Research Council's (MRC's) clinical trials of folic acid for preventing neural tube defects in newborn babies are ready to start within weeks, but the council is waiting for final approval of its plans from the Scottish Home Health Department.

The trials are controversial because they involve giving placebos to a double-blind control group comprising pregnant women who have already given birth to a child with a neural tube defect (NTD) such as spina bifida. There have been fears that if any of the placebo group gave birth to a second child with NTD they could bring a court action in the event of folic acid proving effective. Scottish law in this area is different from English law, and the Scottish authorities are looking hard at the wording of the advice to be given to potential volunteers. Dr Nicholas Wald, who is to coordinate the trials, says the information being provided is "pretty comprehensive".

Earlier this year, Professor J.A. Davis, who was chairman of the trials steering committee, resigned because of reservations about the trials' feasibility. Up to 4,000 volunteers may be required for an adequate assessment of folic acid, and there are doubts about whether this total can be reached in a five-year trial, partly because of adverse publicity. All of the volunteers will already have given birth to one NTD child. Questions were asked in parliament after MRC announced its decision to conduct the trials, which are to take place in the United Kingdom, Australia and Israel.

Previous studies suggesting that folic acid taken in early pregnancy may prevent NTD have the weakness that the study groups were self-selected. In addition, the effect of folic acid was not clearly distinguished from that of other vitamins (see

Nature 300, 396; 1982). MRC has taken the view that the matter can be settled only by a trial with women allocated at random to treatment and control groups. A committee has been set up to monitor the trial, which can be called off if early results show a clear effect.

The Helsinki Code, adopted by the World Medical Association in 1975, re-



quires that the interests of individual patients should always be put before possible future benefits to humanity. MRC's own ethical guidelines appear to support the principle, and the new chairman of the trials' steering committee, Professor Geoffrey Rose, says the trial protocol satisfies this condition. The argument seems to hang on the possibility that folic acid in the high doses to be used in the trials could cause harm, either directly or by raising false hopes about an expensive but worthless treatment. Professor Rose has no comment to make on the Scottish authorities' delay, other than to suggest that it might be a consequence of the recent general election.

Tim Beardsley

Biotechnology

Australian programme revamped

Canberra

THE National Biotechnology Scheme has re-emerged under the new Australian government as the National Biotechnology Program after a couple of months incubation. But whether the difference goes much beyond nomenclature will become clear only when the allocation of funds is announced in August. The Minister for Science and Technology, Mr Barry Jones, hopes the programme will "bridge the gap between laboratory research and implementation of discoveries".

As proposed by the previous government, the National Biotechnology Scheme would administer A\$2.5 million for basic research as well as applied research in projects showing potential for industrial development. Another A\$2.5 million from the Australian Industrial Research and Development Incentives Scheme would be made available to industry specifically for biotechnology. The principal change is that the new programme will abolish the dual support system and integrate all biotechnology support. Hence university projects with a biotechnological bent, as well as the specific commercial opportunities identified by the Department of Science and Technology (DST) at a workshop in November last year, are eligible for support. In spite of the indecision about the total amount to be allocated, DST will shortly be advertising the programme and calling for applications. It may wish to gauge the demand and adjust supply accordingly.

The government proposals are heavily biased towards applied projects, at the risk of neglecting research in molecular biology on which new advances are likely to be based. A DST spokesman said that a minimum condition for award is evidence of industry interest, but industrial involvement would be preferable. Professor Bill Elliott of the Center for Gene Technology, University of Adelaide, is concerned "whether there is sufficient appreciation of the importance of basic research in molecular biology" and considers that a parallel increase of support for basic research may be essential.

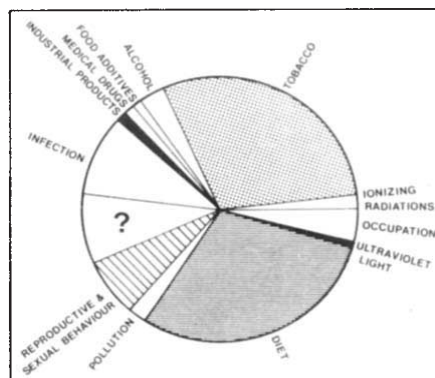
In another move aimed at stimulating the country's biotechnology, the government has decided to introduce legislation allowing Australia to accede to the Budapest Treaty, a patent agreement under the UN World Intellectual Property Organization. According to Mr Jones, "Australia's accession to the treaty will enable industry to have access to microorganisms deposited in any international depository established under the treaty". The need for such access arises because of the technical difficulties of sufficiently describing microorganisms in patent applications. Consequently the present patent law will be changed to enable deposition in an authorized collection to

take the place of a full description for patenting. The legislation will lead to the establishment of an international depository in Australia that might also serve as a national collection for the Australian biotechnology industry.

Vimala Sarma

Cancer causation in booklet form

A NEW booklet published by the epidemiology unit of the British Imperial Cancer Research Fund (ICRF) summarizes the factors which influence the development of cancer. The booklet is based on the book "Causes of Cancer" by Sir Richard Doll (head of the epidemiology unit) and Richard Peto (also from ICRF) which was published in 1981. It takes the criteria drawn up by the World Health Organization in a 1964 report as its definition of extrinsic factors. These cover "man-made or natural carcinogens, viral infections, nutritional deficiencies or excesses, reproductive activities and a variety of other factors



determined wholly or partly by personal behaviour". For each of the environmental factors the booklet assigns a percentage value for the number of cancer deaths in the United States which can be attributed either directly or indirectly to that particular extrinsic factor as compared with the total number of deaths through cancer in any year. The range of confidence with which these figures can be given varies from "highly accurate" in the case of tobacco to "within a factor of two" in the case of occupation and "uncertain but not to be excluded" for food additives. The figures can be misleading, however, as with the effect of alcohol. It appears from the diagram that alcohol accounts for 3 per cent of cancer, whereas alcohol itself does not cause cancer, but instead it enhances the carcinogenic effects of other environmental factors. However, Sir Richard Doll sees the circumstantial evidence for the relative contribution of extrinsic factors to cancer deaths as "sufficient to obtain a conviction in a court of law".

Melanie Kee

Genital herpes

Vaccines show the way

HOPES that a vaccine might one day provide a cure for genital herpes have again been raised by the news that clinical trials of a new vaccine are already under way in the United States, and that a British group is hoping to start trials on another vaccine shortly.

The British group, led by Dr Gordon Skinner of Birmingham University, has developed an acetone-derived subunit vaccine. Although derived from the herpes simplex type 1 virus, which causes oral infections (cold sores), the vaccine is active against the type 2 virus which is responsible for the genital lesions. Tests in volunteers have been encouraging — vaccination protected 97 per cent of partners of known herpes sufferers from attack, and 75 per cent of patients who had themselves suffered one attack were protected from recurrence by three injections of the vaccine. This latter finding is important since it suggests that even though previous infection with herpes does not impart immunity from future infection, a vaccine might give such protection.

As the Birmingham group is quick to point out, however, such limited "volunteer" trials can sometimes be misleading. There is very often a considerable placebo effect and too much dependence on subjective judgements made by the patients themselves. But, encouraged by the early results, the Birmingham workers have started discussions with the UK Committee on the Safety of Medicines with a view to obtaining approval for a properly controlled double-blind clinical trial; similar talks are under way with the US Food and Drug Administration for permission to conduct a trial on 100 patients at Rush-Presbyterian-St Luke's Medical Center in Chicago. Production of the vaccine in the quantities needed for such trials would be undertaken at the Centre for Applied Microbiology at Porton Down in Wiltshire.

In a clinical trial being carried out on 500 individuals in the Seattle area, the US drug company Merck is also testing a DNA-free vaccine. The bulk of the subjects in this trial are the healthy partners of patients who suffer from herpes, and it is clearly in these cases that the vaccine is thought to have greatest potential. Dr Laurence Corry, who is in charge of the Merck trials, feels that the vaccine being tested is unlikely to become available for general clinical use, but that if the results of the trials are promising, a new vaccine will be developed.

The possibility of routine vaccination against herpes, however, is likely to be at least 5 years off even if the present trials are successful. The Seattle trials are due to last about 2½ years and it will be at least 3 years

before the British trials are completed. Then it would take perhaps another 2 years for production methods to be perfected and for the necessary product licences to be issued.

As well as the "conventional" approach, the "biotechnological" route to a herpes vaccine is being tried by at least one company. Molecular Genetics Inc. of Minnesota, working with the drug company Lederle, has got as far as producing an immunologically-active chimaeric protein containing a glycoprotein from type 1

herpes simplex virus (see *Nature* 3 March, p.72). The gene for the protein is expressed in the bacterium *Escherichia coli*, but much work remains to be done before a vaccine becomes a practical proposition.

So there is still no cure for herpes and it looks like being some years before one comes along. In the shorter term, however, there is the prospect of new drugs following on from acyclovir, which has already proved effective in reducing the severity of infections, but cannot eliminate the risk of recurrence. **Charles Wenz**

Space Telescope

Delay as troubles mount

Washington

THE National Aeronautics and Space Administration (NASA) confirmed last week that the Space Telescope, still haunted by technical uncertainties, would not be launched until the latter half of 1986. But James Beggs, NASA's administrator, promised a congressional subcommittee that the telescope would eventually conform to all its original performance specifications.

Beggs' testimony contradicted the findings of a report sent to the House of Representatives Appropriations Committee by congressional investigators (see *Nature* 2 June, p.366). The report claimed that dust contamination of the primary mirror, and deficiencies in the fine guidance sensors supposed to aim the mirror at distant objects, had prompted NASA to ask the scientific community to accept a lower standard of precision.

At hearings last week officials from NASA and the Perkin-Elmer Corporation, which is building the mirror, said new studies of the dust particles on its surface had found that 0.2 per cent of the mirror's surface contained particles, a degree of contamination acceptable in the visible range and "marginally acceptable" in the ultraviolet range. But because of the likelihood that contamination will increase in the three years remaining before launch, the mirror will have to be cleaned. Perkin-Elmer conceded that any attempt to clean the mirror would risk damaging its coating. NASA's project office is reviewing possible cleaning methods but is expected to recommend inverting the mirror and blowing the dust away with clean dry air or filtered nitrogen.

NASA officials told the committee that design problems associated with the crucial fine guidance sensors had proved serious enough to persuade the agency to transform the first flight model, which has still not been completed, into an engineering test model, and to start work on an alternative design in case the original concept proved unworkable. Beggs said the ability of the sensors to track a 14.5 magnitude star and maintain a lock on it for 24 hours had not yet been demonstrated, but preliminary tests of the system's Koesters prism interferometers

were said to be "encouraging".

Speaking a day earlier at the opening of the Space Telescope Science Institute in Baltimore, project scientist Robert O'Dell was equally guarded about the prospects for the fine guidance sensors. He said he was confident they would operate with their specified accuracy "at least most of the time". In an interview later, he said that the Lockheed Corporation had found that the continuously moving reaction wheels used to point the telescope caused vibrations at certain speeds which would make the spacecraft tremor excessively.

O'Dell also disclosed new anxieties about the possible implications of the recent discovery that the space shuttle, and other spacecraft, emit a glow while in orbit (see *Nature* 26 May, p.282). Current speculation is that the glow is caused by the interaction of hydrogen on the spacecraft surface with residual oxygen in the space atmosphere. The glow threatens to contaminate any astronomical experiment operating aboard the shuttle. O'Dell said an experiment designed to explore the phenomenon was being planned for next year, and might result in new materials being selected for the Space Telescope.

In evidence at last week's hearings, Beggs acknowledged that NASA had not foreseen the scope of the technical difficulties the telescope project would encounter, but insisted that recent organizational changes — including the establishment of closer links between NASA and the telescope scientists — would enable the agency to complete the telescope successfully by the end of 1986.

He warned, however, that many of the problems had been unavoidable given the pioneering technology the project entailed. And he anticipated new problems when it became necessary to verify the optical integrity of the instrument prior to launch. Because the telescope is designed to operate in zero gravity, the primary mirror will be distorted by the Earth's gravitational field while it is on the ground. NASA intended to devise a number of "limited" tests that could be performed on the ground and hoped to be able to alter the figure of the mirror when it was in orbit.

Peter David

Madagascar

Western scientists back in favour

PROSPECTS for field researchers wanting to study Madagascar's unique fauna and flora appear brighter than at any time in the past decade. The island's government, after years of discouraging Western scientists, is now seeking to enlist overseas expertise to help in its conservation efforts.

The closed-doors policy started with political upheavals in the early 1970s that came to a head with a military coup in 1975. Thereafter the new left-wing government effectively barred the doors to Western scientists by refusing to grant visas for stays of more than 3 months. Overseas scientists were viewed with suspicion and there was a feeling that the country was not benefiting from their investigations. Eight years later, the government has gained more confidence in its sovereignty, and the efforts of a number of individuals — notably M. Barthelemy Vaohita of the World Wildlife Fund's Madagascar branch — have secured a change in direction.

When news of the government's change in outlook began to spread two years ago, the Department of Scientific Research on the island was flooded with more research proposals than it could cope with. The problem of evaluating the proposals was tackled at a meeting in Jersey early this year, organized by Dr Lee Durrell of the Jersey Wildlife Preservation Trust. Biologists and conservationists from six countries, together with Mme Berthe Rakotosamimanana, director of the Department of Scientific Research, agreed to set up an International Advisory Group of Scientists (IAGS) to review research proposals and make recommendations to the Malagasy authorities. IAGS, which started its work in March this year, has already forwarded three recommendations; the first expeditions to apply through IAGS could set out in August.

Although the Malagasy government is anxious to foster research that is partially oriented towards nature conservation, this condition is unlikely to exclude any sound biological research project. Dr Durrell, IAGS's chairman, says the most important condition is that copies of publications resulting from research in the country should be forwarded both to the university in Madagascar and to the government. One recent expedition was also asked to employ a Malagasy technician. **Tim Beardsley**

Correction

In last week's article "Polish pollution: Changes may come at last", the first sentence on p.567 should read: "A plea last autumn by Deputy Premier Roman Malinowski for 'close cooperation' with Czechoslovakia and the German Democratic Republic to save 60,000 hectares of threatened trees in the western province of Jelenia Gora..."

Nuclear Regulatory Commission

More licence for nuclear industry?

Washington

CITING the increased maturity of the nuclear industry, the Reagan Administration has begun to urge Congress to streamline the procedures used by the Nuclear Regulatory Commission (NRC) to license new power plants. A Department of Energy (DOE) bill, the subject of heated Senate hearings, proposes condensing the present two-step licensing procedure into a single stage, curbing the number and scope of public hearings and limiting the ability of NRC to impose new safety requirements once construction of a plant is under way.

In hearings last week, Ellyn Weiss, representing the Union of Concerned Scientists, said it was "unconscionable" for Congress to reduce public oversight of nuclear safety and draw the already blunted teeth of NRC. The most controversial ingredient of the DOE bill is a provision to limit the authority of NRC to insist on design changes once it has licensed the construction of a plant. The industry blames the proliferation of design changes — known as "backfitting" — for many of the delays and cost overruns experienced by nuclear power plants. Under the new bill, NRC would be allowed to impose a backfit only if it could prove that the change would "substantially" enhance overall safety and that the extra cost could be justified. In addition, all backfitting would have to be approved in person by the NRC commissioners — a proposal the commission itself claims will result in serious administrative bottlenecks.

A second major change would do away with the separate hearings conducted by NRC at the building permit and operating licence stage of power plant construction. Permission to operate a new plant would be granted at the same time as permission to build, subject only to later "certification" — by the utility itself — that the plant had been built in accordance with the original plans and would operate safely. Public hearings would be held only at the original licensing stage and would no longer be "adjudicatory", with sworn testimony and the cross-examination of witnesses.

Energy Secretary Donald Hodel, explaining the new procedure, told the committee that by combining the operating licence and building permit, NRC would be able to make public participation more "meaningful" as well as reassure contractors that they would not be prevented from operating power plants that had been built at enormous cost. To qualify for the joint permit, he maintained, contractors would have to provide NRC with more complete design plans early in the licensing procedure, giving critics of the plant "a real target at which to shoot".

Critics of the bill have ridiculed the administration's contention that the

changes would increase opportunities for public participation. In her Senate testimony, Ms Weiss noted that there would no longer be automatic public hearings after a plant had been built and before it was turned on. More significantly, she said, a change in the legal character of any hearings — from an adjudicatory to a "hybrid" hearing — would place "outlandish and onerous" obstacles in the path of members of the public who wanted to intervene at any stage of the licensing procedure.

Under hybrid hearings, intervenors would no longer be permitted to call and cross-examine witnesses unless they could first prove to the commission that there existed "genuine and substantial disputes of fact which can be resolved with sufficient accuracy only by introduction of evidence at a formal hearing". But they would not be allowed, before proving that such disputes of fact existed, to question utility experts or NRC staff. Such a change, Ms Weiss claimed, would result in wasteful litigation to establish whether a disputed fact was "substantial" or could be accurately resolved.

The Union of Concerned Scientists has also vehemently opposed the proposal to limit the scope of backfits ordered by NRC. Weiss told the committee that most examples of backfitting did not result from changes in NRC regulation but from the belated discovery of deficiencies in plant design. NRC Commissioner Victor Gilinsky, in earlier testimony, said that NRC regulations had become more detailed and prescriptive only because of the "uneven" safety performance of the nuclear utilities and their inability to regulate themselves.

Although the administration's bill has received enthusiastic support from the industry, NRC has produced an alternative bill which offers some streamlining of procedures but retains more licensing hurdles and safeguards. It would leave the scope of backfitting to the commission to resolve administratively, and it would retain the right of members of the public to call for a hearing after a plant had been built. Like the DOE bill, the NRC legislation would combine the operating licence and construction permit, but an NRC review of a completed plant would be required before it could start operation.

Dissenting from his NRC colleagues, commissioner Gilinsky said he could not understand how the single-stage licensing procedure could be coupled with a compulsory review of a power plant after it had been built. "So long as the law tells a utility building a plant that it has a licence, and at the same time tells the regulators that it is still up to them to determine the readiness of the plant for operation, someone is being fooled", he said.

Peter David

UK overseas development

Rayner's cuts "flawed"

BRITISH Government plans to scale down the work of four scientific units run by the Overseas Development Administration (ODA) are criticized in scathing terms in a House of Commons Select Committee report published last week. Concluding that the severe staff and budget cuts intended for the four units are based on scrutinies that are "fundamentally flawed", the committee asks the government to suspend its decisions and think again.

The report was compiled by a subcommittee of the Foreign Affairs Committee (FAC) chaired by Labour MP Frank Hooley. The ODA scientific units, which carry out training and research into problems of developing countries, have in the past two years been scrutinized by Lord Rayner, formerly the Prime Minister's advisor on Whitehall efficiency. As a result, scheduled staff levels have already been cut to 75 per cent of the 1979 total, with further reductions planned. And two of the units have been amalgamated. FAC says the Rayner scrutinies were concerned only with costs, rather than efficiency, and that the total cost of the units (£14.3 million this year) is "minute" in relation to their value to developing countries.

One of the units, the Centre for Overseas Pest Research, has already been forced by cuts to abandon its trials of the molluscicide, nicotinanilide, against the snail vector of schistosomiasis just as "success was imminent". This year the centre was

New men at ministry

PRIME Minister Margaret Thatcher's post-election reshuffle, to the surprise of many, left Sir Keith Joseph still in charge at the Department of Education and Science. Two new under secretaries have been appointed, however, Mr Peter Brooke (left) and Mr Robert Dunn. The division of



responsibilities between them has not yet been announced, but it is likely that Mr Brooke will take over from William Waldegrave (now at the Department of the Environment) in the department's dealings with the University Grants Committee. Mr Brooke, aged 49, a graduate of Balliol College Oxford and the Harvard Business School, was vice president of the National Union of Students in 1955-56.

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formally united with the Tropical Products Institute, which specializes in post-harvest storage, to form the Tropical Development and Research Institute. The new institute, which is still seeking a site, is planned to save £475,000 through a "greater concentration of effort" and staff reductions of 10 per cent. FAC says the merger was not warranted by the Rayner scrutinies, which took "no serious account" of the value of training and scientific information the units provide. It says funds for the two units were well spent and that resources for both should be increased. The merger is widely unpopular with staff of the new institute.

Of the other two units, the Directorate of Overseas Surveys, which produces specialist maps and surveys, will cease to exist in its present form and will be merged with the Ordnance Survey. The government's scrutiny by the Rayner Unit found the directorate to be inclined to an "over-artistic" approach to cartography but recognized it as a centre of excellence. The move to Ordnance Survey's headquarters at Southampton will involve staff reductions of 60 per cent, and many cartographers have already resigned. FAC concludes, after consultations with international aid organizations, that the need for surveying and mapping in developing countries is increasing and doubts whether the directorate will be able to sustain its "highly regarded" work if the planned changes are made.

The Land Resources Development Centre (formally a "special" rather than a "scientific" unit) is to have its staff complement cut to 45, from a 1979 figure of 83. FAC says that the reduction will decrease the centre's cost-effectiveness, which it says compares well with the private sector. The centre is concerned with applied land-use research and undertakes training in developing countries.

The government's plans for the four units, which account for 1 per cent of the overall aid budget, are described by FAC as "a short-sighted error for the sake of small and short-term savings"; the damage to the units, which have an "extremely high international reputation", will "probably be irreversible".

FAC endorses the government's aims of increasing efficiency and encouraging the units to act in a more entrepreneurial manner; this would, it says, increase flexibility as well as efficiency. The aims are consistent with the general enthusiasm in government for "privatization" and the Rothschild customer/contractor principle. But FAC says the units must have more reliable central funding and should be permitted to seek additional funds from outside sources if their accumulated expertise is to be protected. It is not known how the government will respond to the FAC report; however, one senior ODA official, said "Almost certainly none at all".

Tim Beardsley

Soviet oceanography

Costly neglect of plate tectonics

SOVIET neglect of plate tectonics could have serious economic consequences, two leading Soviet oceanologists warned recently. In a major article in *Pravda*, Dr Andrei Monin, director of the Institute of Oceanology of the Soviet Academy of Sciences and Dr Leonid Brekhovskikh, secretary of the Academy's Department of Oceanology, Atmosphere Physics and Geography, accused the Ministry of Geology of making insufficient use of recent advances in tectonics. Moreover, they said, the Ministry of Higher and Specialized Secondary Education needed to update university courses in geology, which dealt only superficially with tectonics.

By the time the article had appeared in *Pravda*, Dr Brekhovskikh was aboard the research ship *Akademik Mstyslav Keldysh*, leading a major expedition with an acoustic monitoring programme aimed at developing rapid acoustic methods for the location of iron and manganese nodules on the seabed. So far, sea-bed exploration has been left almost entirely to the initiative of the Academy of Science's "Commission on Problems of the World Ocean" — which coordinates the efforts of the Institute of Oceanology, the four earth-sciences institutes, and also the Far Eastern Centre of the Soviet Academy and the Institute of Geological Sciences of the Ukrainian Academy. But the main initiative in under-sea exploration has come from the oceanologists — largely due to the reluctance of certain leading geologists, notably Dr Vladimir Belousov, to accept the theory of plate tectonics. This divergence of opinion was carefully glossed over by Monin and Brekhovskikh with a call for "greater creative cooperation" between oceanologists and geologists, and an allusion to the work done by Soviet oceanologists in the development of plate tectonics.

During the past decade, Soviet oceanologists have, indeed, made a number of

notable advances, including the 1976 expedition of the *Dmitrii Mendeleev*, which yielded unique rock specimens from layers 2 and 3 of the oceanic crust, the magnetic survey of the Baltic by the vessel *Zarya* in 1981 and the tectonic mapping of ore occurrence and recovery of iron-manganese nodules in the neighbourhood of the Owen fracture by the *Akademik Vernadskii* in 1979. Very little such work, however, has been organized from the geologists' side — one notable exception being the *Bellinghausen* and *Gidrolog* expedition of 1979, which was a joint venture of the Institute of Geology of the Ukrainian SSR and the naval hydrographic service.

The use of submersible manned and remote-controlled craft has played a major role in these ventures. These are generally multipurpose vehicles, which combine the recovery of bed samples with the photography and video-recording of marine life. They include the *Argus*, which can operate at depths of 600 m and glides over the bottom on ski-supports, and the *Pisces*, which can operate to depths of 2,000 m, the *Manty* and *Krab*, which are equipped with special manipulators, and the *Okeanolog*, which has electrohydraulic grabs capable of removing up to 20 kg of bed samples at a time, and fitted with special engines enabling it to "hover". However, although the Soviet Union can boast of more than 120 research ships (not counting almost the same number of specialized vessels of the hydrometeorological and fisheries services) it has fallen badly behind as regards research submersibles. In all, say Monin and Brekhovskikh, there are some 200 such craft in the world — but only "twenty-plus" of these belong to the Soviet Union. The "further development of earth sciences and the needs of [Soviet] industry" they conclude, requires the considerable expansion of the submersible "fleet" in the nearest future. **Vera Rich**



Akademik Vernadskii, veteran of a 1979 hunt for manganese nodules

X-ray astronomy

Future looks rosy for UK

BRITISH X-ray astronomy, it seems, is basking in a ray of sunshine, whatever clouds may be gathering over the Science and Engineering Research Council (SERC, see *Nature* 4 May, p.5) that supports the subject. Exosat, the European Space Agency's X-ray observatory, is just beginning operation in orbit, while almost simultaneously it appears that SERC has agreed to commit a sum "substantially less than £10 million" to yet another X-ray satellite, the German-led trilateral Rosat. The third partner to the DM300 million (£75 million) satellite will be the United States. Rosat is due for launch on space shuttle number 60 in July 1987.

Dr Joachim Trümper, director of the Max Planck Institute for Extraterrestrial Physics in Garching that designed and is building Rosat, says Rosat is two to three times cheaper than had it been built and launched by ESA and is tremendous value.

Within six months of launch, it will have surveyed the X-ray sky at 1,000 times the sensitivity of the previous whole-sky map (by UHURU) and will then "with luck" perform one to two years of observations on selected objects. Rosat will increase the number of known X-ray sources by a factor of 50, Trümper believes, and will have five times greater sensitivity for quasars than even the biggest ground-based optical telescopes.

Meanwhile, Exosat remains the only X-ray observatory aloft. European Space Agency scientist Brian Taylor, holding the first, hour-old Exosat maps of X-ray source Cygnus X-1, said on Monday that Exosat "looks healthy and is working". One proportional counter on one of two identical telescopes had failed and would be switched off "for some considerable time" until the ground staff could determine what was wrong, but other instruments had shown cosmic radiation backgrounds an order of magnitude lower than had been feared, "extremely low and extremely steady", contradicting early Apollo estimates, said Taylor.

Exosat is three times smaller in focal length — and hence spatial resolution (colour) — than the Einstein observatory and has a smaller collecting area, but is better than Einstein in spectral resolution and in the observation of shorter wavelengths.

Exosat also has a remarkable orbit — out beyond the radiation belts (hence the fears about cosmic rays) to perigee at 191,000 km, compared with Einstein's circular 450-km orbit. This is an historical relic: Exosat was planned well before Einstein, when imaging X-ray telescopes were poor at best and observations were made by collimation. At that time astronomers envisaged locating X-ray sources by lunar occultation, for which an eccentric orbit gave a large apparent motion of the Moon, thus covering some 20 per cent of the sky

with possible occultations. In the event, occultation will hardly be necessary.

But the long orbit will still be useful: the satellite will be controllable in real time, remaining above the radiation belts for some 80 hours on each orbit, thus enabling rapid response to X-ray flares — and to the unexpected.

Robert Walgate

Lüst for ESA

Munich

PROFESSOR Reimar Lüst, president of the elite German research organization the Max-Planck-Gesellschaft (MPG) for the past 12 years, is to be the next director-general of the European Space Agency (ESA). He hopes to convince reluctant member states that ESA's mandatory science budget should be bigger.

Lüst conveys a diplomatic and gentlemanly manner, but this merely conceals the "dynamic" man beneath, his German colleagues say. Sir Hermann Bondi, who was director-general of the European Space Research Organization when Lüst was its scientific director (1962–64), thinks Lüst will be an outstanding director-general.

Lüst is a naval man: in 1943, at the age of 20, he was shot out of a U-boat in the Atlantic and picked up by a British ship. He learned his physics in an American prisoner-of-war camp. He likens ESA's membership to a convoy: it cannot move faster than the slowest ship and cannot turn faster than the biggest.

It is clear, he says, that "the scientific demand in Europe is much greater than ESA can provide". He hopes that governments will see this.

ESA research satellites are not unduly expensive, he believes — although leading space researchers within the MPG say they can be two or three times the cost of launches outside the agency. "I can't confirm the figure", says Lüst. After all, "the same industries build the satellites". But Lüst is "interested to look into this". As for the failures of Ariane, he argues these were just development problems. On communications satellites, where a commercial battle rages in Europe, ESA "must formulate a balance" between small and large states, he says. The objective of ESA is "the technological development of Europe . . . my main priority is to have a coherent programme in research and application".

One little problem he will face when he moves to Paris to take up his job in July next year is that he does not speak a word of French. "My scientific language is BE — broken English" he says. He believes that in science and technology "we should speak English, and not French and not German" — not an attitude that will go down too well in proud France. However, he promises to attend a few French lessons.

Robert Walgate

EEC research

Commission set to push ahead

Stuttgart

EUROPEAN Community government leaders at their summit meeting in Stuttgart again failed to discuss the philosophy behind Community Research Commissioner Etienne Davignon's plans to revitalize Europe's research efforts in the face of stiff competition from Japan and the United States. But the fact that the summit communiqué made only a passing reference to joint research and development activities is unlikely to keep Davignon from pushing ahead with his ambitious new industry-oriented research strategy.

The subject was covered in just one paragraph of the communiqué, which reiterated the government leaders "determination to develop and make more effective Community action in research, innovation and the new technologies with a view to facilitating cooperation between enterprises". There was also a suggestion that the Commission should come up with ideas for "improving the international competitiveness of enterprises". So at least some of Davignon's ideas found their way into the communiqué, and the Commission can now feel free to devise concrete proposals for encouraging the development of biotechnology and telecommunications at a European level.

The first fruits of the Commission's philosophy on research are to be seen in Esprit, the European Strategic Programme for Research and Development of Information Technologies (see *Nature* 9 June, p.461). Pilot phase contracts are now being signed between the European Commission and various European research units.

But for Davignon and the Commission, joint research is only the beginning. The long-term aim is much closer industrial cooperation in areas such as telecommunications where the costs of developing sophisticated new systems are becoming too great for individual countries to bear. What is needed, Davignon states, is "a gradual transfer of powers and means from member states to the European Community institutions".

Davignon knows, however, that it is too early to come up with operational ideas for the European Community to take over some of the roles of the national telecommunications administrations. Governments are increasingly unwilling to give up national powers. So Davignon has asked European Community leaders to appoint a panel of senior officials "who would be able to commit their governments" on the issue. The panel would discuss such matters as how strategic technological options could be decided at Community level, how European industry could cooperate on pre-competitive research and development and how a free choice of suppliers

best be ensured.

The panel, which ideally should come up with preliminary conclusions before the end of the year, would also look into possibilities of setting up a European telecommunications body, placed under Commission authority. Commission sources admitted the ideas were given "a lukewarm reception" by Community governments.

Better received, and less radical, were Davignon's ideas on biotechnology. Davignon pleads for an extension of the 1982 programme, which was focused on agriculture and food, to the health sector. Research would again be equally financed by the Commission and by European industry.

But Davignon's ideas once again exceed the narrow framework of research and development. For Davignon, industry's access to raw materials must be improved. But Community governments are unlikely to follow Davignon on this point: opening up the market to imports of cheap farm produce like starch, a basic requirement of the biotechnology industry, would upset the already fragile and politically sensitive Common Agricultural Policy.

Geert Linnebank

Europe and US to fuse on fusion?

Brussels

THE European Commission wants to develop transatlantic cooperation in research on thermonuclear fusion and has asked member states for permission to negotiate a formal cooperation agreement with the US Administration.

A spokesman for the Commission said the US authorities had welcomed plans to exchange information and staff working on tokamak confinement vessels, alternative magnetic confinement technologies and inertial technology.

The idea of this cooperation was first launched on the eve of the Versailles Western economic summit in talks between Research Commissioner Etienne Davignon and President Reagan's scientific adviser, George Keyworth. A tentative agreement to institutionalize it was reached in March, but Community member states have yet to endorse the plan.

Europe currently leads the field in research on fusion reactors based on the magnetic plasma confinement principle, generally thought to be the most promising approach. Approximately 90 per cent of Europe's fusion research resources are invested in the development of the Culham-based Joint European Torus (JET) giant tokamak, which is expected to become operational later this year, and related projects. But Europe lags behind the United States in alternative technologies and fuel (tritium) handling.

Geert Linnebank

Polychlorinated biphenyls in human tissue

A REPORT published recently by the US Environmental Protection Agency on the levels of polychlorinated biphenyls (PCBs) in human adipose tissue shows that while almost all Americans now have detectable levels of the compounds in their tissues, very few have above the 3 parts per million (p.p.m.) level arbitrarily designated by the report as "high". The figure clearly shows the impact of the regulatory legislation that was introduced in 1976 controlling the "manufacture, processing or distribution" of PCBs. Before this date the number of people with "high" levels had been steadily increasing, but since 1976 this level has dropped dramatically so that now only one per cent of Americans have PCB levels above 3 p.p.m.

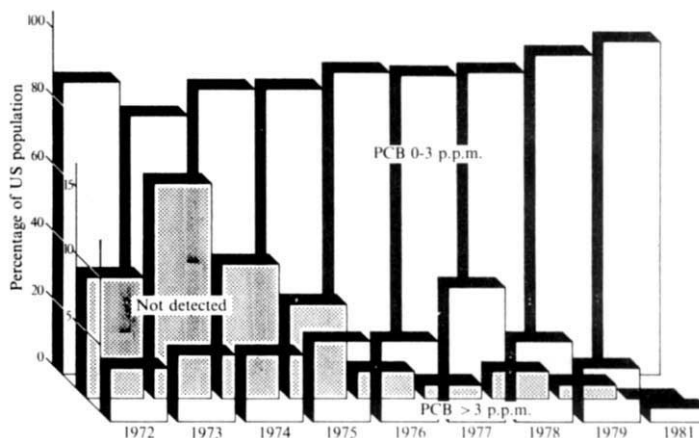
The report lists two caveats — first that the survey was conducted on cadavers

which may not be representative of the general population, and second that only urban populations were investigated. Since much of the controversy over PCBs has involved their inclusion in herbicides this second proviso may mark a significant shortcoming.

The increase in background levels of PCB in adipose tissue, and, to a lesser extent, in human milk, is due at least partly to their use in electrical equipment, chlorinated hydrocarbons being used as a dielectric in transformers and capacitors.

Dr F. Kutz, head of the field department on whose data the report was based, believes that "as a result of certain exemptions from the 1976 Toxic Substance Control Act around 600 million pounds of PCBs are still in existence, either in storage or in electrical appliances".

Melanie Kee



Czechoslovakia

Misplaced effort

PROGRESS in the scientific and technical development of the country was reviewed in last week's meeting of the Communist Party of Czechoslovakia. The official report, delivered by Presidium member Milos Jakes, painted a gloomy picture, the "key problem" being the "slow and inadequate" implementation of research results in practice. Shortly before the meeting, however, the party daily *Rude Pravo* had come up with a new explanation of the lag — inefficient deployment of funds.

According to *Rude Pravo*, the excuse most often proffered by industrial enterprises failing to innovate is that of insufficient funds. However, it went on, a recent survey by the finance ministry had found that some 200 to 300 million crowns a year (£12.5–£19 million) set aside for research and development is going unspent. Often, it appears, the funds are not made available on time so that the planned innovation cannot be started. On the other hand, and particularly when the development programme has not been properly prepared,

"random actions" occur, with funds being misappropriated and diverted to purposes "which have nothing to do with research and development". Sometimes, *Rude Pravo* noted, the funds have been spent on developments whose ultimate economic benefit has never been established.

This somewhat novel criticism found only a brief echo in Jakes' presentation to the party meeting: a call for the better use of the 19,000 million crowns (£1,200 million) annually allotted to developing science and technology (4 per cent of the national income) to be concentrated on the "most decisive tasks" (including energy-saving technologies, microelectronics, the chemical industry and biotechnology).

Both *Rude Pravo* and Jakes suggested that most of the problems could be overcome with better labour discipline, but while the paper called for "the more consistent application of sanctions" to enterprises where there were shortcomings, Jakes concentrated on building up the research base with properly-motivated cadres, the need for better designers, technologists and development engineers, and the need to improve "moral" and financial incentives.

Vera Rich

Are publishers doing their job?

SIR — You ask “who will write more books?” (*Nature* 294, 17; 1981). Here is one depressing answer. With the four books I have published (in 1951, 1968, 1971 and 1982) I encountered no real difficulties in having them accepted for publication. Since then, however, my attempts to interest scientific publishers in further review material have run into every imaginable difficulty.

The first of these books (*Mosquito Behaviour and Ecology: Reviews of Progress*) was submitted to a UK publisher who had expressed interest in the outlined proposal. There were long delays in obtaining comments from the referees I had been asked to nominate and even though these were favourable, the publishers decided — five months after submission of the manuscript — to reject the idea on the grounds of “marketability”.

The manuscript was then submitted to another UK publisher who had reacted encouragingly to the outline. Here again there were long delays in obtaining comments from referees so that after five months I withdrew the manuscript. By this time a US publisher had expressed interest in the project, and agreed last November to publish the book.

This might appear to be a happy ending, but a final obstacle has arisen which has proved insuperable. The large numbers of charts and figures, carefully and painstakingly re-drawn at the publishers request to standards which have been acceptable in the past, have been rejected as not being in “camera-ready” condition. The publishers advise that these should be re-done by a professional artist, presumably at my expense. As there is no way in which I can pay for such an expensive and unforeseen item, the contract has been cancelled by the publishers.

In the case of a second proposed book (*Pesticide Impact on Stream Macroinvertebrates*), the idea has proved acceptable to a continental publisher but this acceptance carries a sting in the tail in that the manuscript must be submitted in “camera-ready” form for photo-offset reproduction. It would be almost impossible for a single author without secretarial help and proper equipment to comply with these requirements. The alternative proposed by the publishers is for them to process the manuscript into a camera-ready condition, and then to debit me to the extent of royalties on the first 1,000 copies.

The effect of all these processes is that anything up to a year may elapse between completion of a manuscript and its final acceptance by a publisher, with a further period of anything up to two years before publication. Not only is the author virtually working for nothing during this period, but these delays make nonsense of any hope, or claim, that a review deals adequately with “recent advances”. This time

lapse is particularly ironical when photocopying facilities could make available copies of reviews within weeks of completion.

These events throw a great deal of light on the query — or the plea — “who will write more books?”. The combined result of all these obstacles, delays and demands is effectively to gag the communication of material which can justly claim to provide the “scholarly reviews in the scientific literature” required.

R.C. MUIRHEAD-THOMSON
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Animal sexing patents

SIR — The news item “Techniques for sexing embryos now possible”¹ was interesting but the statement “Hitherto, the sex of a calf fetus could not be known until birth . . .” should be corrected. In fact, the first calf to have its sex diagnosed as an embryo was born on Christmas day, 1975. That heifer had had a very small piece of trophoblast removed by microdissection before transfer about 2 weeks after fertilization and the sex of the tissue had been determined by chromosomal analysis without harming the calf which develops from the embryonic disk². Since then, calves have even been born from embryos sexed by chromosomal analysis of a few cells aspirated from morula stage embryos still within the zona pellucida³ and, more recently, from morulae which have been microsurgically bisected⁴, allowing one half to be transferred while the other is cytogenetically sexed. Many of these developments have been recently reviewed⁵.

The major advance stemming from the work of Genetic Engineering Inc. is the possibility of sexing embryos at an age when they can still be successfully frozen. It seems, though, regrettable and ironic that such important findings were, because of patent applications, reported without scientific detail or discussion during the same week that over 600 members of the International Embryo Transfer Society were meeting within a few miles of Denver.

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A weighty issue

SIR — Your leading article on lead in petrol (*Nature* 21 April, p.641) is both ill-informed and contradictory. You embrace the conclusions of the Royal Commission on Environmental Pollution¹, but then go out of your way to discredit groups such as CLEAR which gave evidence to the Royal Commission and whose public activities ensured that the government responded quickly to the recommendations made in the report. What you fail to realize is that many of the points made by the commission are precisely those that CLEAR has argued for in the past fifteen months.

Thus, we have always accepted that lead pollution is a multi-source problem, but we have also argued that “the largest single controllable source of lead for the population as a whole is lead in petrol” (see paragraph 7.2 in the report).

We accepted that evidence of lead's harmful effects in the general population may not be conclusive, but levels typically found in the population today are very close to levels known to be toxic (see 5.23).

We accepted that the contribution of lead in petrol to lead in dust and to lead in food may be difficult to quantify precisely, but since lead is a cumulative poison, we argued that it cannot make sense to allow environmental burdens to go on increasing indefinitely (see 1.6).

Finally, the edited proceedings of the CLEAR symposium² were submitted to the Royal Commission in their entirety. If the writer of your leading article is able to identify any statement in those proceedings which is either “dishonest” or a “shameless exaggeration” of the evidence, then I would be interested to learn of it.

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- Royal Commission on Environmental Pollution, Ninth Report, *Lead in the environment* (HMSO, London, 1983).
- Lead versus Health, Sources and Effects of Low Level Lead Exposure* (eds. Rutter, M. & Russell Jones, R.) (Wiley, Chichester, 1983).

Oxbridge entrance

SIR — In editing my note on “Who goes to Oxbridge?” (*Nature* 26 May, p.278) a confusion was introduced. The figure of 14.6 per cent of the 1,068 maintained school candidates (men + women) placed in Class I in their final examinations at Cambridge last year was to be compared not with “10.9 per cent of firsts for the independent school girls” but with 10.9 per cent of the 862 candidates of *both* sexes from the independent schools. That percentage was somewhat depressed by the disappointing 4.4 per cent of firsts for independent school girls, as compared with 8.8 per cent for girls from maintained schools, the difference between male candidates from the maintained and independent sectors being considerably less than that. C.B. GOODHART
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An embarrassment of peptides

Molecular biology is revealing an extraordinary array of new, potential hormones. The problem is how to determine their physiological functions, if any.

As molecular biology sweeps through pastures new, it tends to generate a mixture of despair, excitement and frustration in roughly equal parts. The despair is of those who see the limelight shifting to the molecular upstarts. The excitement stems from the amount of new information that is generated in short order by the new techniques. And the frustration comes in trying to translate the molecular information back into physiological terms.

So much was evident from two recent meetings on neuroscientific topics where the molecular approach held sway. First was a small gathering in Hamburg* and then this year's major symposium at Cold Spring Harbor, which will be reported in a later issue of *Nature*.

The Hamburg meeting illustrated just how effective the molecular approach is by comparison with its predecessors in establishing the structure of the precursor molecules from which neuropeptides/hormones (the distinction is ever more blurred) are derived. Many years ago it became clear that peptides are cleaved from much larger precursors — the erstwhile "big" or even "big, big" forms of the peptide. But to establish the structure of a precursor was a long, hard grind involving endless analytical biochemistry combined with radio-immunoassays and biological assays of countless samples.

Even when a precursor emerged from the quagmire, there was always the question of whether it was the original precursor or merely an intermediate. Molecular biology has turned the tables. The approach now is to home in on the messenger RNA for what is, by definition, the ultimate precursor, convert it into DNA and sequence it. A moment's consultation with the genetic code then allows the deduction of the amino acid sequence of the precursor.

What emerges, invariably, is that the sequence of the known peptide is flanked on one or both sides by other peptides. Sometimes the existence of the other peptides is already known. For example, Dietmar Richter's laboratory has shown that in its precursor, arginine-vasopressin is followed by neurophysin II which was already known to be somewhere in the precursor. And neurophysin II is followed by a third peptide that was already known as a consti-

tuent of the pituitary but not as a molecular confederate of arginine-vasopressin and neurophysin.

More frequently, previously unknown peptides are revealed by sequencing a precursor messenger RNA. For example, mouse nerve growth factor is preceded by three new potential peptides (*Nature* 302, 538; 1983). And, in Hamburg, both William Rutter and Axel Ullrich described the existence, to judge mainly by a repeated cluster of regularly spaced cysteine residues, of about eight epidermal growth factor-like peptides preceding the factor itself in its huge precursor (see p. 722 of this issue).

The excitement generated by such sequencing lies not only in the potential discovery of new peptides with biological function. For once cloned precursor DNA becomes available, it can be used to scan tissues for the presence of its equivalent messenger RNA by the technique of *in situ* hybridization. Olivier Civelli described in Hamburg how that technique had been used to detect messenger RNA from the pro-opiomelanocortin gene in several areas of the rat brain. Such an approach, still in its infancy, promises to clear up many a controversy generated by claims of the synthesis of a peptide in an organ, tissue, cell or neurone based only on its presence there (and often detected by inadequate means). Only if the relevant messenger RNA is also present can it be certain that the peptide has been synthesized *in situ* and not imported from elsewhere.

Some would go further and argue that the presence of messenger RNA for a peptide precursor is tantamount to proof that the peptide will be present, given that messenger RNA is seldom made when not needed and tends not to stay around for long once made. That, however, is to push the molecular biological approach too far because even if the precursor is made from the messenger, it cannot automatically be assumed that production of a given peptide follows.

That is where the frustration engendered by the molecular approach begins. Tempting though it is to assume that the numerous potential peptides spotted in a precursor sequence are really produced, proof is another matter. There are, however, strong clues in the presence of potential cleavage sites in precursor sequences; indeed, it is essentially by the cleavage sites that the presence of discrete, new peptide moieties is recognized. Com-

monly the cleavage site is a pair of basic amino acids but it is clear that not all such pairs are cleaved and that less easily identifiable sites can be cleaved. Moreover, there are several well documented ways in which a cloven peptide can be further processed. Reliably predicting the end products of a precursor is therefore no child's game.

Predicting the function of the presumed end products is almost impossible. Who is to say that the second gastrin-like peptide in the gastrin precursor, the two extra glucagon-like peptides in the glucagon precursor or the eight vaguely similar peptides that precede epidermal growth factor have any function? Perhaps they are evolutionary debris. Quite probably they bear sufficient resemblance to the "authentic" peptide to exhibit some activity in any appropriate biological test. But does that imply a real function?

With peptides that bear no relationship to any other, the problem is where even to start. The number of such peptides is increasing rapidly through molecular biology but the problem, of course, predates the technique. At Hamburg, for example, Michel Chretien could do no better than to attribute some mitogenic activity to a large new pituitary peptide isolated and partially sequenced by classic means. And Chica Schaller was at a loss to explain the presence and function in mammalian brain and gastrointestinal tract of the same small peptide that she has painstakingly shown to be responsible for promoting the growth of *Hydra* head.

So how can the functions of peptides best be determined? One possibility is to turn to lowly organisms like the sea slug, *Aplysia*, or the fruit fly, *Drosophila*, which have several advantages, not least that they are amenable to molecular genetics. For those whose curiosity extends to mammals, current approaches to the determination of function are crude. One is to inject the peptide into animals or on to cells simply to observe the results. Another is to test the effects of injecting antibodies against the peptide.

The proper interpretation of that type of experiment and refinements of technique will depend upon the unfashionable skills of physiology. Immensely attractive though molecular biology must be to young scientists, we cannot afford to be without a new generation of bright physiologists able to pick up from where the molecular approach runs out. **Peter Newmark**

*Biochemical and Clinical Aspects of Neuropeptides: Synthesis, Processing and Gene Structure", 29 May-2 June, Hamburg-Blankensee.

Particle physics

Will the noise at CERN come out tops?

from Frank Close

WHEN the history of 20th century physics discoveries is written, two years will stand out. One is the *annus mirabile* 1932. The other is 1983, in which physicists at CERN have already made discoveries that rival those of the past and soon may even surpass them.

The year 1932 saw the arrival of the Cockcroft-Walton accelerator and the discovery of the neutron and the positron — the first example of antimatter. Antimatter is now used as a tool in the gargantuan modern descendant of the early accelerators — the superprotonsynchrotron (SPS) at CERN, in which antiprotons and protons collide head-on and annihilate one another, fleetingly creating a fireball of energy from which have now emerged the W^+ and W^- particles, the carriers of the weak nuclear force, first reported in January 1983 (*Nature* **301**, 285 and 287; **302**, 478). This already was a monumental discovery: as the W mass has been determined more precisely, it has been found to agree nicely with the predictions of the Weinberg-Salam theory uniting the electromagnetic and weak forces. This pivotal theory also requires the existence of a massive analogue of the photon, known as the Z^0 and with a predicted mass of 94 ± 2.5 GeV; and in the first week of June, the discovery of the Z was announced with a preliminary value for its mass of 95.2 ± 2.5 GeV! These discoveries have profound implications for the nature of matter and the behaviour of forces, and may already contain the cryptic signature of yet another manifestation of the fundamental interactions predicted by electroweak theory: the so-called top quark.

The W and Z particles transmit forces among particles of matter in the same way that the photon transmits electromagnetic forces. The forces transmitted by the W are most familiar in generating radioactive decays and for nearly 50 years have been known as the 'weak' forces. The discovery that the W and Z have masses slightly above 80 and 90 GeV now proves that the forces they transmit are intrinsically of the same strength as electromagnetic forces — they seem to be weak at low energies only because they are carried by such massive entities. Thus the electroweak force has two manifestations: the familiar form transmitted by (uncharged) photons and another transmitted by massive (uncharged) Z^0 .

At energies comparable to the Z mass the two forms of force are of comparable magnitude, but at low energies the Z^0 force is barely noticeable, swamped by photon-transported electromagnetism. Nature does not contain 'charged photons' that could transmit a similar more prominent force and mask the W^+ and W^- effects.

β -radioactivity, in which the uncharged neutron is transmuted to a charged proton, revealed the electrically charged version of the 'weak' force and thus we set off in pursuit of understanding. From this day on we have a loftier perspective; at high energies all these forces have the same strength: only at low energies are the $W^\pm$ and Z^0 effects frozen out and the force consequently weakened.

Once you extract a new secret from nature new discoveries may be expected to follow soon. Such may be the case with the W at CERN: there are growing suspicions that the background 'noise' in the experiments that revealed the Z^0 signal may also contain evidence for the decay of the W particle into top and bottom quarks — a symmetry that would reinforce the growing support for the electroweak theory (UA1 collaboration, 5 June; 1983).

In particular, an important ingredient in the theory of the electroweak force is the hypothesis that it acts on quarks and on leptons in essentially the same way. This is in line with the observation that the six known leptons respond to the weak charged force (W^+ and W^- interaction) in characteristic pairs, and four of the five known varieties of quark also respond in pairs. It is natural to want to complete this symmetry by postulating the existence of a sixth quark ('top' quark) that will pair with the fifth ('bottom' quark) when interacting with $W^\pm$ (*Nature* **271**, 406).

For example, one of the lepton pairs consists of the electron and a neutrino: a W can decay directly into this pair and be detected (indeed this was how the W was discovered). The top and bottom quarks form a pair that can analogously be produced directly when a W decays (technically, this yields a top quark and a bottom antiquark, or vice versa depending on whether it is W^+ or W^- that decays). According to the electroweak theory W particles should decay into the quarks more often than into leptons (electron and neutrino). The actual rate depends on the mass of the top quark but given that some 40 examples of the decay to leptons have been seen, many examples of decays into top-bottom quarks should be lurking in the data.

This has been stressed in particular by Barger, Martin and Phillips (*Phys. Lett.* **25B**, 343; 1983) who have pointed out that such decays should exhibit a characteristic signature enabling them to be recognized rather easily. The massive W should decay into top and bottom quarks which set off in opposite directions, preserving overall momentum. The relatively light bottom quark should then shower into a highly collimated jet of particles such as pions and kaons. In the opposite hemisphere, the

massive top quark should move slowly and sometimes undergo radioactive decay, yielding a rather broad shower of particles including an energetic electron or positron. Barger *et al.* accordingly advocate searching for events in proton-antiproton annihilation containing an electron with a high transverse momentum component normal to a narrow jet and accompanied by a broader jet.

The background noise mentioned in the Z paper seems to be of this kind. Analysis of the accumulating sample will confirm if this is indeed what is happening and, if so, determine the mass of the top quark. The fact that top quarks have not yet been produced in electron-positron collisions shows that they are more massive than 20 GeV; if they are being observed in the decay of an 80-GeV-mass W then top quarks must be somewhat less than 70 GeV (probably much less or phase space would be too limited for us to have seen any).

Confirmation of the electroweak theory also raises some wider issues. Anthropic arguments (Carr and Rees *Nature* **278**, 605; 1979) suggest that we might not be here if the (low energy) strength of radioactivity were different. It has its empirical strength because the W is at 80 GeV, not 8 or 800. What natural law requires that W and Z have this fortuitous mass? Are they elementary or composite? Do they gain their masses through Higgs mechanism and if so do consequent Higgs particles exist, awaiting discovery? There is no consensus of opinion on the masses of Higgs particles, or even on whether they are elementary; and here we meet a great mystery.

Parity violation and the properties of Higgs mesons are injected into the theory *ad hoc*; there must be some profound principle of nature that governs them, but at present it is hidden from us. If Higgs particles are discovered, perhaps among the decay debris of Z^0 (Z^0 radiating a photon and transmuting to a neutral Higgs particle is one possibility that has been suggested), then study of their properties may help us crack a great unsolved puzzle — what is the origin of mass, and where do the mass scales come from?

The distinct scales of 100 GeV for W and Z (weak force) — 10^{15} GeV for the force postulated to cause proton decay and 10^{19} the scale of quantum gravity (the Planck mass) — are mysterious. The discovery of W and Z , great as it has been, may turn out to be merely the key that will unlock much deeper truths. Time will show the real significance of 1983. □

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Carcinogenesis

Testing for carcinogenicity and the problem of pseudocarcinogenicity

from Francis J.C. Roe

MANY oncologists breathed a sigh of relief when, over a decade ago, emphasis was switched from screening chemicals for cancer chemotherapeutic potential to testing selected chemicals for carcinogenic potential. Not only were the prospects of prevention more promising than those of effective treatment, but extrapolation from laboratory animals to man seemed more valid for carcinogenesis tests in which intact animals were simply exposed to the test chemical via an appropriate route than for cancer chemotherapy screens based on inhibition of the growth of transplanted tumours in animals. Recently, however, it has become increasingly clear that the interpretation of carcinogenicity tests in animals is not necessarily simple and that reappraisal of the methodology is overdue.

When the Carcinogenesis Testing Program was started by the National Cancer Institute (NCI) in the USA, great emphasis was put on standardizing the test procedures and for most tests Fischer 344 rats and B6C3F₁ hybrid mice were used. The primary purpose of tests was to see whether selected chemicals had any potential for carcinogenicity at any site, and since for almost all known carcinogens tumour incidence increases with dose, it was deemed logical to expose animals continuously to maximum tolerated doses (MDT) throughout most of their lives. Mainly because of the risk that the selected putative MTD would in practice prove excessive, a second dose level equal to 1/2 MTD was included in the study design.

Through this approach an unexpectedly large number of chemicals never previously associated with cancer have produced positive results for one or more sites in rats and/or mice. In an attempt to defend these chemicals many toxicologists have questioned the reliability of extrapolating to man from positive results obtained by exposing animals to extremely high doses that overwhelm normal metabolic pathways and defence mechanisms. To this the 'hawks' have responded that for carcinogens such as ionizing radiation there is no evidence for the existence of any threshold. Therefore, they argue, a positive result in response to high exposure implies some risk at low exposure and the most sensitive test is one that involves maximum exposure. Those who actually set up the original NCI testing programme claim that it was never meant to be more than a screen for possible carcinogenicity. Positive results were to be regarded as no more than a trigger for properly designed dose-response studies going down to human exposure levels and for studies of possible mechanisms.

It is now generally understood that carcinogenesis by penetrating ionizing radiation is not a universally suitable model for carcinogenesis by chemical agents, partly because potentially genotoxic chemicals or the genotoxically active metabolites of chemicals may, in practice, never reach target DNA and partly because some chemicals which enhance cancer risk do so by a mechanism that involves no damage to DNA and no evidence of mutation. The number of alternative non-genotoxic mechanisms by which chemicals can enhance cancer risk is legion. For some, a threshold has been demonstrated, for others a threshold probably exists, and for yet others, there may be no threshold. An understanding of mechanisms is obviously very helpful in determining whether there is likely to be a threshold dose and, if so, what it is.

But it is snags of yet other kinds which have recently been highlighted by Haseman¹ in a review of patterns of tumour incidence in a series of 25 tests in Fischer 344 rats carried out under the National Toxicology Program (NTP) which took over the NCI Carcinogenesis Testing Program. Taking together the results of the 25 tests, all of which involved exposure to the test chemical admixed with food, Haseman found that the number of treatment-associated significant ($P < 0.01$) increases in organ-specific tumour incidence was approximately equalled by the number of comparable decreases. Furthermore, in about two-thirds of the tests more high-dose than control animals of one or both sexes survived the full 2 years of study. Thus survival was often significantly improved by treatments which NTP concluded were carcinogenic. Most of the positive findings in the tests were for liver tumours whereas the apparently beneficial effects of treatment were mainly on mammary and lymphoreticular neoplasms.

Citing evidence in the literature that reduced dietary intake and reduced weight gain are associated both with reduced spontaneous tumour incidence and, although he does not mention it, with improved survival, Haseman¹ postulates that the treatment-related decreases in tumour incidence are consequential on treatment-related inhibition of body weight gain. To test this theory he analysed the NTP data for any association between tumour incidence and body weight gain during the second year of the experiment. Had he been aware of the recent report by Ross *et al.*² he might have paid more attention to the effects of treatment on body weight at the start of experiments, since those

authors found that differences in diet during the few weeks after weaning had profound and permanent effects on subsequent tumour risk. Nonetheless, Haseman found that treated male rats with deficits of more than 18 per cent in body weight gain during the second year of study had fewer incidences of tumours of the pituitary, thyroid (C-cell), adrenal medulla and pancreas (islet cell) than controls. In low-weight-gain females, reduced incidences of mammary fibroadenoma and particularly pituitary tumours were seen. In neither sex was reduced weight gain associated with reduced incidence of lymphoreticular neoplasia. However, four out of five compounds which significantly enhanced liver tumour incidence, simultaneously significantly reduced the incidence of lymphoreticular neoplasia.

Of the 25 substances reviewed by Haseman, it is likely that fewer than a third had the potential to act as genotoxic carcinogens. Thus most of the 17 positive results probably depended on non-genotoxic mechanisms, 7 of them affecting endocrine glands (Leydig cell, thyroid C-cell, pancreatic islet cell) or hormone-controlled tissues (uterus). With the possible exception of lymphoreticular neoplasia, all 17 examples of beneficial effects of treatment on tumour incidence were also of endocrine (Leydig cell) or hormone-controlled (uterus, mammary gland, preputial gland) tissues. Both sex hormone status and nutritional status affect lymphoreticular neoplasia in mice, maleness and diet restriction reducing the risk^{3,4}. The data reviewed by Haseman for the F334 rat provide no evidence of similar effects in the rat, but a mechanism in which hormones play a part has by no means been ruled out.

The design of the standard carcinogenicity test in rodents is based on the assumption that untreated control animals are representative of unexposed humans. It is, alas, abundantly clear that this is not the case because, as the animals get older, they exhibit a very high incidence of mixed endocrine disturbances associated with incidences of endocrine tumours which are not encountered in humans (see, for example, ref.5). Clinicians impatient for new drugs may well be shocked to learn that promising drugs are 'killed' because they

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significantly increase the incidence of one or other kind of endocrine tumour in rats that spontaneously show such serious disturbances of general hormonal status.

Why are untreated laboratory animals so abnormal from a hormonal viewpoint? Over-provision of an over-nutritious diet is doubtless partly responsible, but it may well be a mistake to equate this simply with body weight gain or calorie intake. The beneficial effects of diet restriction may be secondary to hormonal changes associated with being faced for a part of each day by an empty food basket rather than with an overall reduction in calorie intake⁶, or with the enforcement of celibacy in the conditions of continuous sexual stimulation that exist in animal rooms where there are separately housed animals of both sexes. If these factors are important, then one must expect non-specific effects on endocrine status and tumour incidence from factors such as increased or decreased palatability of diet and from treatment-related changes in libido or sex appeal.

It would also not be unreasonable to ask 'why haven't those responsible for funding and carrying out carcinogenicity tests paid more attention to getting the conditions right?' 'Why have they seemingly taken no steps to evolve ways of maintaining laboratory animals into old age in normal hormonal status?' There are several reasons: for example, too few testers think strategically; too many evaluators think in numbers rather than of mechanism; changes in methodology would devalue background data accumulated at great expense. Of course, none of these reasons is sound and what we urgently need are micro-methods for assessing the hormonal status of living animals. These would facilitate research on mechanism and help us to distinguish between true carcinogenicity and pseudocarcinogenicity dependent on laboratory artefacts. □

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Magnetism

Ferromagnetic domains without decoration

from John N. Chapman

It is almost eighty years since Weiss predicted the existence of domains in ferromagnetic materials and more than sixty years since the experiments of Barkhausen indirectly confirmed it. Soon afterwards, decoration techniques, pioneered by Bitter and still in use today, provided a direct and often beautiful picture of surface magnetic domain structures in a wide variety of ferromagnets. The interest in domain physics stems largely from the influence of the domain structure on the overall magnetic properties of ferromagnets. This in turn makes different materials suitable for use, *inter alia*, as electromagnets, permanent magnets or as information storage devices. To understand why a particular material is suitable for one or other of these applications, information is required not only on the surface domain structure but also on the mobility of domain walls, their detailed structure and their interaction with, for example, crystalline defects. The decoration technique, in which the surface of the specimen is coated with a liquid containing a colloidal suspension of magnetic particles which tend to agglomerate where domain walls intersect the surface, cannot provide all this information. Thus there is incentive to seek alternative methods capable of overcoming some of the limitations of the Bitter technique.

To this end a number of different experimental methods have been developed and some of the most promising of these are described below.

The rotation of the plane of polarization

of plane-polarized light on reflection from the surface of a ferromagnet (Kerr effect) has been widely used to study surface domain structures at a resolution down to $\sim 1 \mu\text{m}$. Dynamic as well as static effects may be studied by this technique.

More recently scanning electron microscopy (SEM) has been used for a similar purpose¹ and offers the advantage of the simultaneous availability of a wide range of signals containing not only magnetic but also structural and compositional information. Indeed, until recently a serious problem associated with the technique was the danger that any magnetic contrast was liable to be swamped by that arising from other sources. This has been overcome in some cases by using a 'lock-in' technique² which not only reveals the domain structure more clearly but also is particularly well suited to investigating the dynamics of wall motion. However, despite their advantages, neither the Kerr effect nor use of the SEM allows information on domain structures well within the bulk of the material to be determined nor do they have sufficient resolution to allow wall structures themselves to be probed.

The Faraday effect involves the transmission of plane-polarized light through transparent specimens and, like the Kerr effect, relies on the rotation of the plane of polarization to reveal the existence of domains. Recently it has been used with great effect to study both the static and dynamic properties of bubble domains but its use is evidently restricted to transparent

specimens and as with all optical techniques its resolution is limited by the wavelength of light. To overcome this limitation, radiation with a shorter wavelength must be used and transmission electron microscopy (TEM) with electron energies in the range 100–1,000 keV provides an attractive alternative. It has a resolution sufficient to enable the spatial variation of magnetization across a domain wall to be determined and new imaging techniques^{3,4} demonstrated within the last two years greatly simplify image interpretation. The technique is also well suited to the study and identification of defects and so enables their influence on domain walls to be assessed⁵. This should help in obtaining a microscopic understanding of the coercivity of different materials. However, as with the other techniques discussed, TEM does suffer from limitations, the most serious of which is that even in the most favourable conditions it is restricted to specimens $< 1 \mu\text{m}$ thick.

To investigate domain structures within the bulk of specimens too thick for either the Faraday effect or TEM to be used, X-ray and neutron topography may be employed⁶. The former technique relies upon the magnetostrictive effect to reveal magnetic contrast and as such is unsuitable for studying 180° walls. Its use is likely to increase as synchrotron radiation facilities become increasingly available and it is well suited to studying wall-defect interactions and domain structures in antiferromagnets. It is a comparatively low-resolution technique and is best suited to specimens with a low defect density. Neutron topography offers the advantage of yet thicker specimens ($> 100 \mu\text{m}$) but with a further loss of spatial resolution.

A new technique which also depends on the magnetostrictive effect has been developed by Parpia *et al.* and is reported in this issue of *Nature*⁷. It relies on the distortion of adjacent domains in the specimen causing the surface to acquire a rippled appearance which may be viewed in an optical microscope without polarized light. Like other techniques, it cannot provide all the required information, but if used in conjunction with them, it will help to reveal new facts about magnetic domain structures. This in turn will aid our overall understanding of magnetic properties and the further development of magnetic devices. □

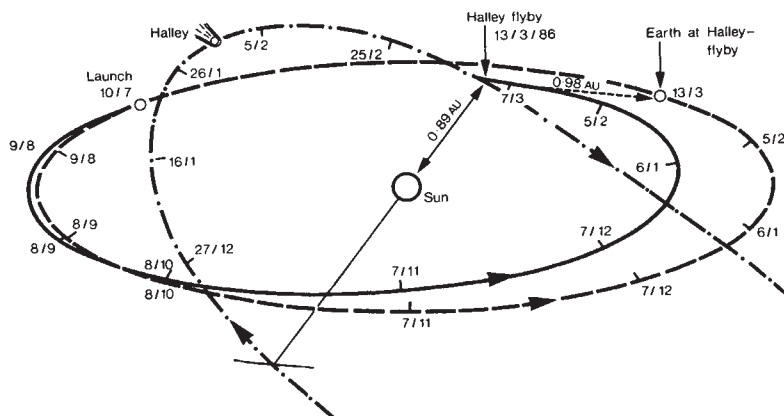
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Astronomy

Giotto spacecraft in danger

from Max K. Wallis



Reference trajectory of Giotto spacecraft

ESA's interplanetary spacecraft Giotto will fly by comet Halley in March 1986 on a trajectory that will bring the spacecraft within a few hundred kilometres of the comet nucleus. It is intended that it should pass through the scientifically interesting, relatively dense part of the dust cloud surrounding the nucleus. This will inevitably disturb the spacecraft's attitude, and could lead to a loss of information. An important consideration in the design of Giotto has therefore been to identify the exact cause of the disturbance so that appropriate countermeasures can be taken.

Giotto will be spinning nominally at 15 r.p.m. and is equipped with a two-element shield to protect its vital equipment from damage by dust particles impacting at a relative velocity of 69 km s^{-1} . The outer 1-mm aluminium 'bumper' shield causes small particles to fragment and vaporize, while the inner Kevlar sheets, some 25 cm behind it, protect against the expanding debris.

It was first thought that impacts of 10–100-mg grains would present the greatest danger, for at 69 km s^{-1} these might only partially fragment, smash through several millimetres of shielding and perhaps disrupt crucial components. But it is now realized that the mission is endangered by still smaller grains causing tilts of the axis by more than 1 degree, the width of the transmitted radio-beam. There is no rapid re-orientation mechanism, so once contact with Earth is lost the spacecraft would pass far through the comet and lose much of the scientific data before the transmission link could be restored.

The present strategy is to fly as close to the comet as feasible without substantial risk of exceeding the 1-degree perturbation. So it is important to calculate that risk carefully. One aspect of the calculation is inevitably uncertain, since the actual dust particle fluxes and sizes will only be known from studies carried out once comet Halley has brightened in the months before the en-

counter, and indeed will only be accurately determined by the Giotto mission itself.

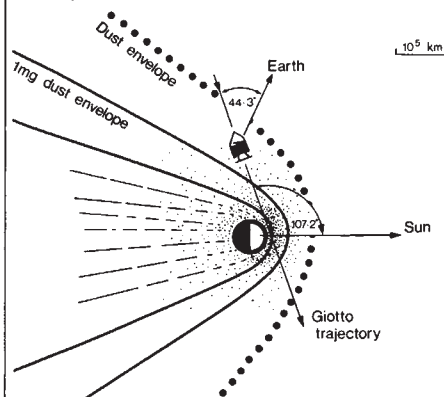
A second difficulty is that impulses due to the 69 km s^{-1} impacts cannot be simulated in practice. When ESA scientists Coupe and Dean first studied the problem (*ESA J.* 7, 15; 1973), they assumed that impacting particles simply give up their momentum to the spacecraft. Totalling the impulses over a theoretical distribution in size, they found that the rare largest particles cause the greatest perturbation. They therefore devised a Monte-Carlo calculation to simulate stochastic impacts ranging over size and position and found tilts of the axis of around 1–2 degrees and infrequently of several degrees.

But impacts at 69 km s^{-1} do not simply deliver momentum. As shown by experiments with dust particles moving a factor of 10 more slowly, they cause vaporization of a much greater mass of material which, if it escapes anisotropically, can exert many times greater an impulse than the initiating momentum. Tony McDonnell of the University of Kent conducts such experiments and, extrapolating from his group's and others' results, has pointed out that a multiplication factor of around 40 is in order. This perhaps applies only to particles of less than a microgramme in mass, for larger ones penetrate to the inner shield or even further, where the vapour they release does not penetrate primarily forwards.

As technical factors limit experimental particle speeds to about 10 km s^{-1} or exceptionally 20 km s^{-1} , laser impacts are being studied as a way to simulate the 69 km s^{-1} impacts. Recent laser experiments at SERC's Rutherford-Appleton Laboratory (W.M. Burton *Adv. Space Res.* 2, from the COSPAR Symposium on 'Impact processes on solid bodies', 1982) and France's Ecole Polytechnique (J.-P. Bibring *et al. J. Phys.* 44, L189; 1983) have demonstrated crater formation analogous to that from dust impacts. The impulse in some cases is

sufficient to penetrate 0.5-mm aluminium sheets. If the scaling factors relating them to solid particles can be established, the high-power laser bursts will be the best way of simulating hypervelocity impacts.

Another feature of Giotto is that its shield and exposed instruments present asymmetric surfaces to the oncoming dust. The ESA scientists decided that because the spacecraft rotates, the net effect of unbalanced impacts would be zero. I have challenged this as conceptually flawed. The situation is analogous to an unbalanced spinning top, or more accurately, to an unbalanced gyroscope in a head wind: the asymmetric torque induces a precession of the rotation axis, the precession angle growing with time. For the spacecraft spinning in free space, the mathematical problem is a classical demonstration of the use of Euler's equations: the angular momentum vector wanders in inertial space at the same time as the spacecraft axis precesses. While Giotto rotates once every 4 seconds, the precession which depends on anisotropy in inertial moments has a 35-s period. I calculate that on the planned spacecraft trajectory through comet Halley, the angular momentum vector tilts by up to 1.5 degrees and combines with a precession of ± 0.5 degrees, depending on the actual momentum factor. Thus the mission appears at risk not only from possible milligramme-sized impacts, but also from



Geometry of the Giotto encounter with Halley.

the 'rain' of non-penetrating microgramme dust.

The scientific controversy needs to be resolved quickly. As the spacecraft design is well advanced, it will be necessary to decide whether to meet the problem, for example, by attaching counterbalancing sections to the shield to improve symmetry. The higher-mass grains that penetrate the outer bumper and generate puffs of vapour between the shield sections have not yet been taken into account, as their modelling and calculation present difficulties — it may be that laser impacts on a scale model can help quantify and test practical solutions. □

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Tumour virology

p53 protein and control of growth

from Guil Winchester

THE altered expression of cellular proteins in transformed cells is by now a well documented phenomenon. The cellular function of many of these proteins is, however, virtually unknown. At a recent meeting* one of the first to be discovered, p53 protein, started to look less shadowy. New data, some recently published and some presented at the meeting, suggest that p53 may well be a cell-cycle protein. Whether it is actively involved in the switch from a resting to a growing state or whether it is a passive consequence of rapid cell growth is still not clear; but the technology to answer that question should soon be available.

p53 was first detected in mouse cells transformed by the DNA tumour virus simian virus 40 (SV40), as a host cell protein tightly bound to large-T antigen, the SV40 gene product required for the initiation and maintenance of transformation¹. A similar complex has been found in cells transformed by adenovirus, between p53 and a protein coded for by the *E1b* region of adenovirus². Since this region is also responsible for the transforming capability of the virus this suggests that the complex may be involved in a route to transformation common to two unrelated DNA tumour viruses.

p53 is also present in elevated amounts in a wide variety of other transformed cells, which include spontaneous teratocarcinomas³, and tumour cell lines induced by chemicals, radiation and RNA tumour viruses⁴. Metabolic labelling and immunoprecipitation experiments have shown that transformed cells seem consistently to express more p53 than their untransformed counterparts. However recently developed immunometric assays, which are capable of detecting steady-state levels of p53 protein, have revealed very large differences in the amount of p53 present in different transformed lines. SV40- and adenovirus-transformed lines consistently have the highest levels, and there are a number of transformed lines where the amount of p53 appears to be as low as in normal cells (ref. 5 and A. Levine, SUNY, Stonybrook). Primary human tumours so far show the same variability (L. Crawford, ICRF, London). These differences in steady-state p53 levels do not correlate with tumorigenicity, but it must be remembered that existing assays measure the concentration of immunologically reactive molecules and this does not necessarily reflect the concentration of functional p53 molecules.

One possible explanation for the higher

levels of p53 in transformed cells is that it is connected with rapid cell growth *per se*. This idea is supported by experiments showing that normal lymphocytes which have been stimulated to proliferate with the mitogen concanavalin A show increased levels of p53 labelling⁶. Now two new pieces of evidence suggest that p53 may be a cell-cycle protein, involved in the regulation of cell proliferation.

The first comes from microinjection experiments reported last October⁷. Using quiescent nontransformed 3T3 cells which had been induced to enter a round of cell division by serum stimulation, Baserga's group showed that the injection of an anti-p53 monoclonal antibody into the cells was able to inhibit DNA synthesis. Inhibition was only effected if the antibody was injected within 2 hours of serum stimulation.

Also working with 3T3 cells, Nancy Reich (SUNY) has looked at the expression of p53 following serum stimulation. p53 mRNA, detected on Northern blots using a nick-translated cDNA probe⁸, starts to rise 7 hours after stimulation and this is followed by a rise in the rate of synthesis of p53 protein at about 10 hours. DNA synthesis is first detectable at 12 hours and the rate of synthesis of p53 protein is already falling as DNA synthesis peaks in S phase.

On this evidence p53 is synthesized as a late G₁ protein. Whether it is acting in G₁, or earlier in G₀ as the microinjection experiments seem to imply, is still unclear. It was recognized⁷ that if p53 levels rose after serum stimulation the failure of antibody to inhibit DNA synthesis at the later times might simply reflect the inability of the amount of antibody injected to mop up the increased levels of protein.

A protein involved in the regulation of the cell cycle might be expected to have a short half life^{9,10} and in untransformed 3T3 cells p53 has a half life of 25 min. This is in marked contrast with the half life of p53 in SV40-transformed cells (SV3T3) which is more than 24 h (ref. 11). The increase in the half life is at least partly due to the stabilization of p53 by binding to large-T antigen. It seems that the increase in half life is responsible for the increased levels of p53 since the levels of p53 mRNA in SV3T3 cells are no higher than in the parent nontransformed cells¹¹.

New data on the regulation of p53 levels in two other transformed lines with elevated levels of p53 was presented at the meeting (N. Reich). In the mouse teratocarcinoma line F9 and in the methylcholanthrene-transformed cell line Meth A, the levels of p53 mRNA are twice as high as in the 3T3 and SV3T3 cell lines, so here at least part of the increased levels of p53 seems to be the result of increased levels of

transcription. In F9 cells induced to differentiate by treatment with retinoic acid and dibutyryl cyclic AMP the level of mRNA drops to about one-tenth of its previous level. These results were obtained using a p53-specific cDNA probe⁶, and largely confirm the earlier estimates of mRNA levels based on the levels of translatable message in an *in vitro* system^{11,12}.

The amount of p53 protein in the F9 and Meth A cell lines appears also to be controlled by post-translational mechanisms. The half life of p53 in these cells is 3 and 5 hours respectively, but if the cells are pulse-labelled and then treated with cyclohexamide to inhibit any further protein synthesis the newly synthesized p53 protein becomes stabilized, with a half life of more than 24 hours. These results argue for the existence of a regulatory element for p53 which itself has an extremely short half life. There is an intriguing difference between transformed and untransformed cell lines in that cyclohexamide does not extend the half life of p53 in nontransformed 3T3 cells.

A possible clue to the function of the p53 complex in adenovirus-transformed cells was reported at the meeting by Levine. The adenovirus *E1b* protein 58K, which in transformed cells is found complexed to p53, is capable of forming an alternative complex. In productively infected cells 58K is complexed to a 25,000-molecular-weight adenovirus protein (25K) coded for by the *E4* region. Since there are 25K mutants available these may throw light on p53 function, although as Levine pointed out the two proteins are not necessarily equivalent. "This is biology, not geometry." It is however encouraging that adenovirus mutants in 58K and 25K have the same phenotypic effect, implying that they have a common function which is connected with the complex.

To those in the field the major excitement of the meeting was the fact that several groups now have cDNA clones specific for p53 (P. Chumakov, Institute of Molecular Biology, Moscow¹³; M. Oren, Weizmann Institute, Rehovot; J. Jenkins and S. Benchimol, Marie Curie Foundation, Oxted and ICRF, London; A. Levine). The ICRF group has direct amino acid sequence data that both give the frame

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and confirm the identity of the clones (K. Leppard).

The first results to emerge from the use of these structural probes indicate that there are two p53 genes. This raises interesting questions about the degree of homology between them and whether they are both functional. As it becomes available, structural data should also clarify the relationship between p53 and the protein found in Epstein-Barr virus

transformed cells, thought at one stage to be equivalent to p53 but now clearly different (ref. 14 and D. Lane, Imperial College, London). The availability of structural probes will also help enormously in the task of unravelling the regulation and function of p53 in normal and transformed cells. □

Guil Winchester is an assistant editor on Nature.

Geophysics

Magnetospheric plasma composition and the solar cycle

from Stanley W.H. Cowley

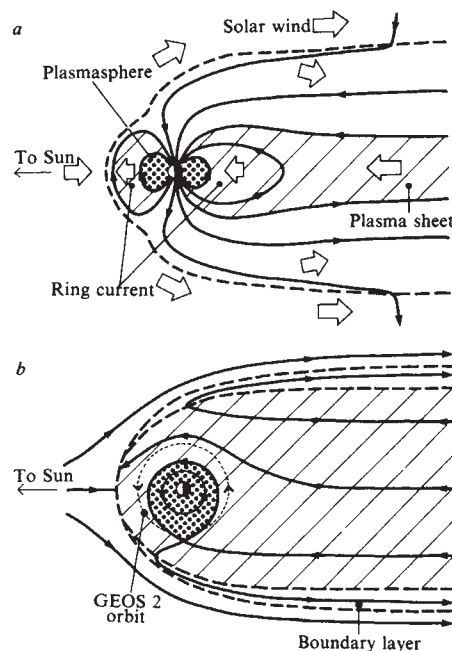
WITHIN the Earth's magnetic field lines that cross the equatorial plane at distances beyond ~ 5 Earth radii is a tenuous (~ 0.1 – 10 cm $^{-3}$) but energetic (~ 1 – 100 keV) trapped particle population termed the 'ring current' plasma. Until recently it was believed that this plasma originated mainly from the solar wind, entering across the boundary of the Earth's magnetosphere and being transported into the inner regions by large-scale convective flows. From the known composition of the solar wind it was then predicted that the ring current would consist primarily of protons (H^+), with a few per cent doubly charged helium ions (He^{2+}) and, of course, charge-neutralizing electrons. It was also recognized, however, that cold (~ 1 eV) plasma would flow out of the Earth's ionosphere into the nightside magnetosphere where it could be accelerated along with the trapped solar wind plasma in the hot ring current. This plasma was also predicted to consist mainly of protons, since the heavy ions which dominate the low-altitude ionosphere (for example singly charged oxygen, O^+) were expected to be closely confined by gravity, but it was expected to be distinguishable from solar wind plasma by traces of He^+ , the difference in charge state between solar wind and ionospheric helium reflecting the difference in source temperature.

It was therefore very surprising that early ion composition experiments flown on spacecraft detected not only large quantities of ionospheric H^+ and accompanying He^+ in the inner ring current, but also occasional large fluxes of kiloelectron volt O^+ . Indeed, during strong magnetic disturbances O^+ could become the dominant ion in this region $^{1-3}$. These terrestrial ions have since been found to be directly in-

jected into the magnetosphere at energies of tens of electron volts to ~ 10 keV from altitudes of a few thousand kilometres above the auroral-zone ionosphere, the acceleration being due to the resonant wave absorption or field-aligned voltages associated with the auroral process. Further energy input occurs during transport within the magnetosphere.

It is now known that the composition of the plasma in the ionospheric acceleration region varies over the 11-year solar cycle, becoming denser and richer in heavier ions at solar maximum compared with solar minimum, in response to changing solar extreme ultraviolet (EUV) emissions which produce ionization, heating and dissociation of molecular species in the high-altitude atmosphere. This has led scientists to examine whether the variations are reflected in the properties of the magnetospheric ring current plasma, and the results of the first detailed investigation of this question have recently been published by Young *et al.* 4 . (See the figure).

Young and his colleagues used GEOS 1 and 2 data from the inner ring current for the interval May 1977 to March 1981, which encompassed the solar maximum in late 1979. The basic unit of information used was the 3-h averaged ion number density measured by the University of Berne/MPI Ion Composition Experiment in the energy band 0.9–15.9 keV per charge for each of the five ions routinely detected above limiting densities of $\sim 10^{-3}$ cm $^{-3}$ (H^+ , He^+ , H^{2+} , O^+ and O^{2+}). These data were then divided into six groups of equal geomagnetic activity based on the *Kp* index and further averaged over each month. The *Kp* division ensures that observations for different time periods can be compared in conditions of similar magnetic distur-



Earth's magnetospheric plasma flows and resulting plasma populations in the noon-midnight meridian (a) and in the equatorial plane (b). The plasmasphere region indicated by dots is dominated by cold ionospheric plasma. Hatching indicates the hot plasma sheet and ring current populations; these populations are not physically distinct but the terms are usually applied to the nightside magnetic tail and the nearer-Earth quasi-dipolar field regions respectively. In a, the solid lines are magnetic field lines and the broken line is the magnetopause boundary. Solid arrows indicate the predominant plasma flows. In b, the solid lines are plasma streamlines, showing division of the flow into two regimes. The outer solar-wind-driven cyclical flow is very variable in magnitude, however, increasing with increasing magnetic disturbance, and the boundary between the flow regimes moves in response.

bance, and further enables disturbance-dependent composition changes to be studied. To examine the dependence on solar activity, the density data were correlated with monthly averages of the 10.7-cm solar microwave flux ($F_{10.7}$). This flux serves as a crude indicator of the solar EUV emission which itself is not directly measurable on the ground.

The results obtained by Young *et al.* show that each of the above ions varies in a characteristic way with respect to $F_{10.7}$ (solar EUV) and *Kp* (magnetic disturbance). All the ions originating in the ionosphere (H^+ , He^+ , O^+ , O^{2+}) show distinct increases with $F_{10.7}$, these being more marked with increasing ion mass as expected on the basis of the ionospheric composition variations mentioned above. The only ion exclusively of solar wind origin, He^{2+} , shows no such correlation with $F_{10.7}$.

The extent of the composition changes due to changes in solar activity can be appreciated by comparing the number density averages for the months of May 1977, when solar activity was low, and November 1979 at or near solar maximum in both periods,

Changes in ion composition with solar activity

Month	Total ion number density (cm $^{-3}$)	% Ion composition					Mean ion mass (a.m.u.)
		H^+	O^+	He^+	O^{2+}	He^{2+}	
May 77	0.29	93	5	1	0.5	0.5	1.9
Nov 79	0.63	48	44	5	3	0.2	8.2

magnetic activity was low ($Kp < 20$) (see the table). In the first interval, protons (H^+) are the dominant ion; the ionosphere is clearly an important source as indicated by the low He^{2+}/H^+ ratio of ~ 0.01 compared with usual values of ~ 0.05 in the solar wind. In November 1979 the absolute H^+ and He^{2+} average densities were little altered, while the densities of the heavier ionospheric ions, O^+ , He^+ and O^{2+} , had each increased by at least an order of magnitude, so that the average number densities of H^+ and O^+ became comparable. Thus during solar maxima heavy ionospheric ions, notably O^+ , are important not only during magnetically disturbed periods, but also during quiet conditions. An O^+ -dominated magnetosphere therefore occurs at these times.

Turning to correlations with magnetic disturbance, the GEOS 2 solar maximum data used for this study show relatively small (~ 60 per cent) increases in both H^+ and He^{2+} from quiet to active conditions (typical H^+ values being $\sim 0.4 \text{ cm}^{-3}$), while O^+ increases markedly from ~ 0.1 to $\sim 0.6 \text{ cm}^{-3}$. These results reflect a moderately greater injection of solar wind ions into the inner ring current during active conditions, together with increased ion outflow, particularly of O^+ , from the ionosphere. The marked increase in O^+ probably results from localized increases in ionospheric ionization and heating in the auroral zone which occur during magnetic disturbance, as discussed by Young *et al.*, as well as perhaps to an expansion in the area of the ionosphere where accelerated outflows occur. In view of the latter it is perhaps surprising that Young *et al.* find no Kp response at all in the He^+ and O^{2+} densities which remain constant at $\sim 10^{-2} \text{ cm}^{-3}$ levels.

Finally, no evidence has been found for the presence of heavy molecular ions from the lower ionosphere (for example N_2^+ , NO^+ and O_2^+) in the ring current plasma, at levels ≥ 0.3 per cent of the total ion density, indicating that the usual ionospheric heating rates are insufficient to raise appreciable numbers of these ions into the ionospheric accelerator region. During major storm periods, however, large fluxes of these ions have been observed above $\sim 1,000 \text{ km}$ altitudes, so that as Young *et al.* point out, it may then be profitable to search for their presence in the ring current. In fact two intense storm periods in July and September 1982 may already have provided such an opportunity, and the outcome of investigations during these intervals are awaited with interest. $\square$

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Obituary

Ulf von Euler 1905-1983

from Sir William Paton

THE death of Ulf von Euler on 9 March, at the age of 78, breaks a link with a classical period of physiological pharmacology in the 1930s when the foundations of the theory of chemical transmission were laid, along with the development of sensitive bioassay and of drug receptor theory. His own major discoveries were Substance P, with J.H. Gaddum in 1931, prostaglandins (now a large family of substances) in seminal fluid in 1934, and noradrenaline as the final neurotransmitter of the sympathetic nervous system in 1936, for which he was awarded the Nobel Prize in 1970. His papers were always distinguished, compact and crisply focused, but one can think it probable that they all stem in part from the time he spent in 1930-31 working with Gaddum on the pharmacological activity of tissue extracts in Dale's Hampstead laboratory — remembered affectionately as 'F4' by those who worked there. Had Gaddum been alive, one would expect him, too, to have shared the award.

There is little need to develop the significance of the pharmacologically active substances today, although their full physiological and pathological role in the body is still to be revealed. But it is worth considering the methods by which they were discovered, and whether they still have lessons for us.

Although extracts of tissues or body fluids had been long and profitably studied (yielding, for instance, thyroid extract, sex hormones and adrenaline), it was only with the unequivocal isolation of histamine as a constituent of mammalian tissue, and with Loewi's experiments on the release by nerve stimulation of active substances in the heart, that intensive work on such activity was seen to be really worth while. And how primitive the available technology now seems! String, plasticine and the smoked drum; no specific antagonists beyond atropine and ergotamine; no chromatography; impure enzymes; and chemical characterization only possible when enough of the pure substance could be obtained in crystalline form for elemental analysis. Sensitivity to temperature, acid, alkali and proteases, and different solubility in organic solvents were the primary chemical tools. Sometimes skilful use of pharmacological desensitization helped. The other main weapon was careful choice of biological test object; and no doubt a Senator Proxmire of the day could have made due capital out of tests with a tissue mush on the dorsal muscle of a leech or on the rectal caecum of a hen. It was fiddly work, unimpressive to watch, with its own tricky logic, and it needed both a faith in pharmacological specificity

and an experimental mastery of dose-response relationships. Yet it allowed the first recognition of most of the neurotransmitters and active substances worked on today.

One may ask how this was possible. The answer is surely that on the side of the pioneers was what one can now see as almost a biological truism: that if there is some diffusible chemical substance in the tissues that exerts physiologically important effects, then such issues must also contain recognition sites coupled to effector systems mediating those effects. Even if the substance is present in an amount too small by a factor of 10^3 or more for chemical detection, yet if it is biologically effective, the recognition site should have a comparable sensitivity. It is this that led to the rise of the bioassay in the 1930s and maintains it as an experimental technique today.

It is interesting that the capacity for biological detection still commonly runs ahead of chemical detection, although mass fragmentography and (where specific antibody can be raised) immunoassay are rapidly closing or reversing the gap. One must still expect bioassay to be needed for the discovery of many other unknown substances; clumsy it may be, but its great strength remains, that the assay itself incorporates something of the functional significance of the substance, and even if the assay is not perfect, the functional importance remains.

It is, therefore, perhaps timely to let von Euler's work remind us of the importance of the biological response, as the pendulum swings towards chemical techniques. *Mutatis mutandis*, Dale's words in 1933 in his third Dohme lecture are also worth recalling:

"The discovery, in artificial extract from an organ or tissue, of a substance which on artificial injection produces a pharmacodynamic effect provides only a first item of presumptive evidence in support of a theory that the action of this substance plays a part in normal physiology. Much more evidence is required before we can attribute clearly defined functions to such a substance, as we can now do in the cases of histamine and acetylcholine. But even when this is already possible, we have still no evidence to justify the assumption that the substance comes naturally into action in the body in the free condition in which we isolate and identify it in the laboratory after various unnatural chemical procedures."

Few have done more than von Euler to satisfy the demands of Dale's programme. $\square$

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Period doubling and chaos in partial differential equations for thermosolutal convection

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Numerical experiments on two-dimensional thermosolutal convection reveal a transition from periodic oscillations to chaos through a sequence of period-doubling bifurcations. Within the chaotic region there are narrow periodic windows. This is the first example of period-doubling in solutions of partial differential equations. A truncated model indicates that this behaviour is associated with heteroclinic explosions.

A LIGHTLY damped system displaced from stable equilibrium exhibits gradually decaying oscillations. If, in addition, the system is acted on by a small destabilizing force that lags in phase behind the restoring force, the oscillations may grow exponentially with time until nonlinearities become important. While the destabilizing force is sufficiently weak the oscillations will be strictly periodic but, as the driving force is increased, behaviour becomes increasingly nonlinear and there may be a transition to aperiodic, or chaotic, oscillations. One route to chaos that has been extensively studied is by a sequence of bifurcations, at each of which the period doubles¹⁻⁵. Beyond the accumulation point of this sequence the oscillations are typically aperiodic. Period doubling has been found in various experiments, as well as in solutions of difference equations and ordinary differential equations but has not hitherto been demonstrated for partial differential equations.

Thermosolutal convection provides an example of such a system that also has extensive applications (refs 4-6 and refs therein). Consider motion driven by heating from below a layer of fluid (such as water) containing a stabilizing concentration of a solute (such as salt) that diffuses less rapidly than heat. Oscillatory convection can then occur even when the mean density decreases upwards. Oscillations are found in laboratory experiments, though they give way to multilayered convection⁷ if the fluid is heated too vigorously. Previous theoretical studies have shown that there can be a transition from periodic to chaotic behaviour as the rate of heating is increased.

Steady and oscillatory convection

In two dimensions, thermosolutal convection in a boussinesq fluid is described by the nondimensionalized equations^{8,9}

$$\frac{1}{\sigma} [\partial_t \nabla^2 \psi + \partial(\psi, \nabla^2 \psi) / \partial(x, z)] = R_T \theta_x - R_S S_x + \nabla^4 \psi \quad (1)$$

$$\partial_t \theta + \partial(\psi, \theta) / \partial(x, z) = \psi_x + \nabla^2 \theta \quad (2)$$

$$\partial_t S + \partial(\psi, S) / \partial(x, z) = \psi_x + \tau \nabla^2 S \quad (3)$$

where σ is the Prandtl number, τ ($0 < \tau < 1$) is the ratio of the solutal to the thermal diffusivity, and R_T, R_S are respectively the thermal and solutal Rayleigh numbers. The quantities ψ, θ, S are the streamfunction and the deviations of the temperature and solute concentration from the nonconvecting state

($\psi = \theta = S = 0$). We adopt the boundary conditions

$$\psi = \psi_{zz} = \theta = S = 0 \quad \text{on } z = 0, 1 \quad (4)$$

$$\psi = \psi_{xx} = \theta_x = S_x = 0 \quad \text{on } x = 0, \lambda \quad (5)$$

which correspond to periodic rolls.

Linear theory shows that for $R_S > \tau^2(1 + \sigma)R_0 / \sigma(1 - \tau)$, where $R_0 = \pi^4 \lambda^{-4} (1 + \lambda^2)^3$, there is a Hopf bifurcation from the static state to oscillatory convection at $R_T = R_T^{(c)}$, followed by a simple bifurcation at $R_T = R_T^{(e)}$ that gives rise to a branch of subcritical (unstable) steady solutions^{8,10}. This branch eventually turns round and acquires stability, since stable steady solutions with larger amplitudes have been found numerically for $R_T > R_T^{(min)}$ (where $R_T^{(min)} < R_T^{(e)}$) (refs 8-11). The oscillatory branch apparently terminates on the unstable portion of the steady branch where the period of the oscillations becomes infinite^{12,13} (see Fig. 3).

For certain values of the parameters, nonlinear oscillatory solutions of equations (1)-(3) show a transition from periodic to aperiodic behaviour before the end of the oscillatory branch^{10,11}. To understand the nature of this transition we have studied it in considerable detail, using numerical solutions of the system (1)-(5) obtained by finite difference methods^{11,14}. For all these calculations we imposed symmetry about the centre of the convection roll, so that $\psi(x, z) = \psi(\lambda - x, 1 - z)$, and so on, and set $\sigma = 1$, $\tau = 10^{-1/2}$, $R_S = 10^4$. These values were chosen here, as in earlier work^{9,11}, to avoid undue computational effort; nonlinear thermohaline convection ($\tau \approx 10^{-2}$) produces more disparate thermal and solutal structures, which are less easily resolved⁷. The aspect ratio λ was either $2^{1/2}$ (the value that minimizes both $R_T^{(c)}$ and $R_T^{(e)}$ and was used in refs 9, 11) or 1.5 (for convenience in defining a computational mesh) and the mesh intervals were varied, though there were always at least 188 independent mesh points. The bifurcation pattern that we shall describe was unaffected by halving the mesh intervals or by altering λ (though changing λ from 1.41 to 1.5 may displace the positions of specific bifurcations by up to 1.5%).

Period doubling into chaos

When $\lambda = 2^{1/2}$, $R_T^{(c)} = 7,720$ and $R_T^{(e)} = 32,280$. Huppert and Moore^{10,11} followed the oscillatory branch and discovered a bifurcation at $R_T \approx 9,200$. Nonlinear oscillations can be described by plotting some global property, such as the spatially averaged kinetic energy, E , or the Nusselt numbers N_S, N_T that measure the rates of transport of solute or heat across the layer, as functions of time. In Fig. 1a and b, N_S and N_T are shown

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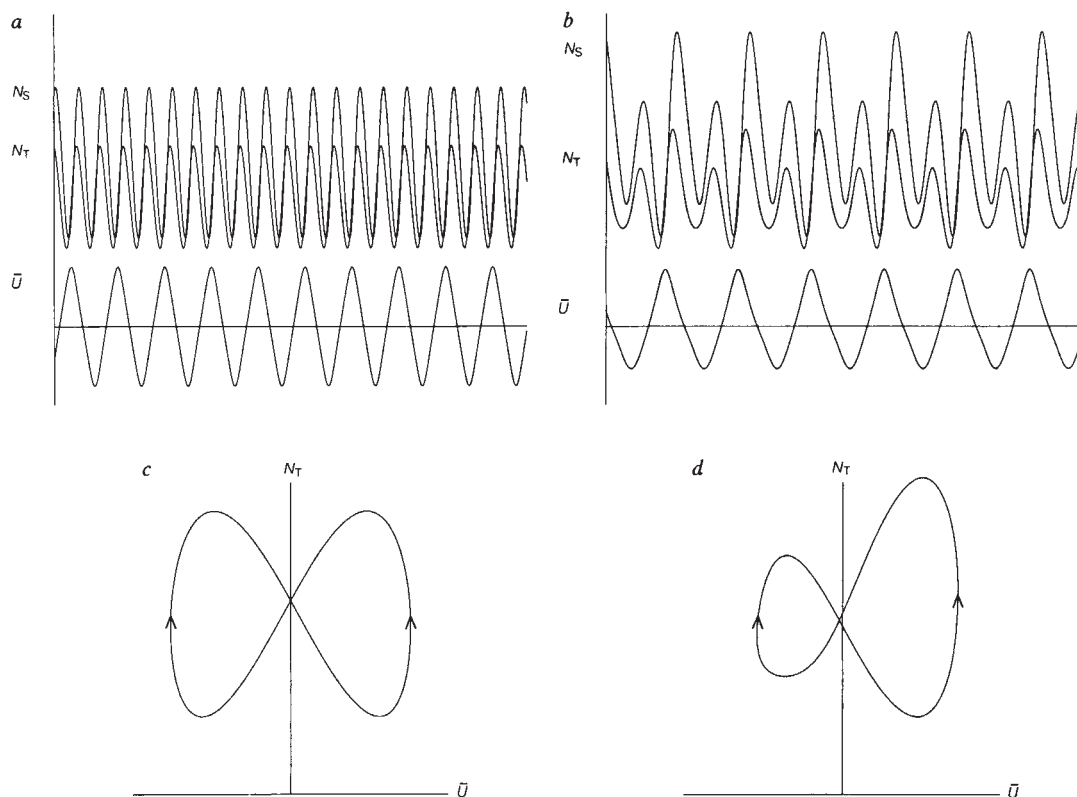


Fig. 1 Symmetrical and asymmetrical oscillations in solutions of the partial differential equations ($\lambda = 2^{1/2}$): plots showing N_S , N_T and $\bar{u}$ as functions of time for *a*, $R_T = 8,600$; *b*, $R_T = 9,800$, with *c*, *d* the corresponding limit cycles projected onto the $\bar{u}$ - N_T plane.

for $R_T = 8,600, 9,800$ respectively, together with $\bar{u}$, the mean value of the horizontal velocity at the base of the cell. N_S and N_T are quadratic quantities (like the kinetic energy) and in Fig. 1*a*, where the oscillations are symmetrical, they vary with half the period of $\bar{u}$. In Fig. 1*b* the oscillations are asymmetrical and N_S , N_T and $\bar{u}$ all have the same period. Thus the first bifurcation is from symmetrical to asymmetrical oscillations¹², as shown by the trajectories in the $\bar{u}$ - N_T phase plane that are plotted in Fig. 1*c* and *d*.

This is followed by a series of period-doubling bifurcations. Figure 2*a* shows a limit cycle in the N_T - N_S plane for an asymmetrical oscillation (period 1), followed in Fig. 2*b* and *c* by limit cycles with period 2 and period 4. Three successive period-doublings have been identified and the ratio of the increments in R_T , $\delta \approx 5$, is consistent with that found for one-dimensional maps¹⁵⁻¹⁷. For $10,150 \leq R_T \leq 10,300$ all solutions were aperiodic but at $R_T = 10,325$ there was a solution with period 4; subsequent bifurcations led to solutions with period 2, followed by asymmetrical and, finally, symmetrical oscillations of period 1, as shown in Fig. 2*d-f*. As R_T increases, there is apparently a complete Feigenbaum period-doubling cascade, followed by an inverse sequence of bifurcations at which the period is halved. The overall bifurcation pattern is sketched in Fig. 3.

Transitions in a simpler system

Similar behaviour has also been found¹⁸ in a truncated fifth-order model due originally to Veronis⁸. The model equations¹²

$$\dot{a} = \sigma[-a + r_T b - r_S d] \quad (6)$$

$$\dot{b} = -b + a(1 - c) \quad (7)$$

$$\dot{c} = \varpi(-c + ab) \quad (8)$$

$$\dot{d} = -\tau d + a(1 - e) \quad (9)$$

$$\dot{e} = \varpi(-\tau e + ad) \quad (10)$$

where $\varpi = 4\lambda^2/(1 + \lambda^2)$, $r_S = R_S/R_0$, $r_T = R_T/R_0$, and so on, are an asymptotically exact consequence of the full system (1)–(5) to $O(a^3)$. (Note that when $r_S = 0$ equations (6)–(8) reduce to the

Lorenz system, which also exhibits double Feigenbaum cascades when ϖ is sufficiently small¹⁹.) The truncated model is not a valid approximation to the full two-dimensional problem when a is finite. Nevertheless, the overall bifurcation structures of both systems are almost identical¹² and the fifth-order system can be used to gain a qualitative understanding of solutions to the full equations. As the value of $|a|$ (that is, of $r_T - r_T^{(0)}$) is increased, the fifth-order model typically exhibits a bifurcation from symmetry to asymmetry followed by a period-doubling cascade terminating in aperiodic solutions. Figure 4 shows some results for parameters corresponding approximately to those of Fig. 2, the modes c and e being linearly related to the Nusselt numbers N_T and N_S . As might be expected, the truncated system has less structure in its limit cycles; there is, however, a complete period-doubling cascade, followed by chaotic behaviour beyond $r_T \approx 13.564$ ($R_T \approx 8,918$). The bifurcation sequence is relatively compressed and occurs at lower Rayleigh numbers for the truncated model, while the oscillatory branch terminates shortly after the accumulation point. Still, the qualitative resemblance between the transitions to chaos in the two systems is close enough to indicate that we are dealing with the same phenomenon.

Heteroclinic explosions

The model system (6)–(10) has the advantage that the steady branch can be found analytically and the eigenvalues describing its stability can be calculated¹². Knowledge of these eigenvalues allows us to understand why chaos occurs. Shil'nikov²⁰ studied a three-dimensional system (that is with three independent variables) containing a homoclinic orbit connecting a saddle-focus to itself. If the eigenvalues at the saddle-focus are s_1, s_2, s_3 , where $s_1 > 0$ and s_2, s_3 are complex conjugates with negative real parts satisfying

$$|\operatorname{Re} s_2| < s_1 \quad (11)$$

Shil'nikov showed that the Poincaré return map for nearby trajectories contains a countable infinity of horseshoes. Each of these horseshoes contains an invariant Cantor set with a countably infinite set of (nonstable) periodic orbits of arbitrarily long periods and an uncountable set of bounded (nonstable)

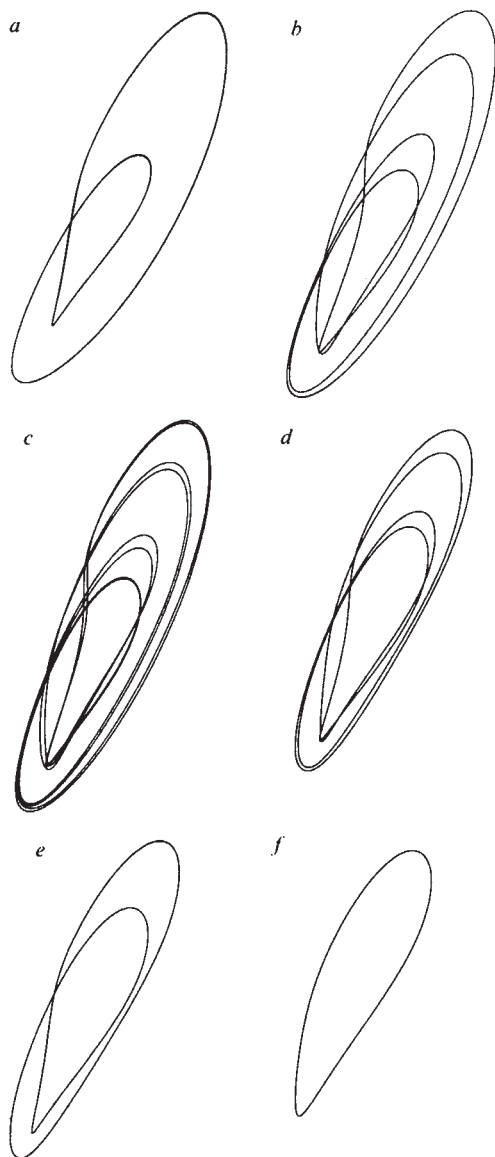


Fig. 2 Periodic solutions with $\lambda = 2^{1/2}$. Limit cycles in the N_T - N_S plane for *a*, $R_T = 10,000$ (asymmetrical period 1); *b*, $R_T = 10,100$ (period 2); *c*, $R_T = 10,120$ (period 4). Further period doublings and an interval of chaos are followed by period halving leading to *d*, $R_T = 10,350$ (period 2); *e*, $R_T = 10,400$ (asymmetrical) and *f*, $R_T = 10,500$ (symmetrical). Orbits are described in an anticlockwise sense.

nonperiodic orbits. Sparrow¹⁹ calls the bifurcation that leads to the creation of so many orbits (with a single horseshoe in the return map) a homoclinic explosion. The Lorenz equations provide good examples of such behaviour¹⁹. In the situation described by Shil'nikov, multiple bifurcations occur as the stability parameter is varied so as to approach the homoclinic orbit, and in small parameter ranges there may be many attracting orbits. In the presence of symmetry, the same behaviour will also be associated with heteroclinic orbits²¹.

The fifth-order system (6)–(10) possesses the reflectional symmetry $(a, b, d) \rightarrow -(a, b, d)$, together with at least one heteroclinic orbit, where the branch of oscillatory solutions meets the branch of (unstable) steady solutions¹². To investigate behaviour at this point, we compute the five eigenvalues s_i along the branch of subcritical solutions that bifurcates from $r_T^{(e)}$, following ref. 22. For $r_T^{(e)} > r_T > r_T^{(\min)}$, s_4 and s_5 are real and negative (that is stable). For small r_s all the eigenvalues are real, with $s_1 > 0$ and $s_5 < s_4 < s_3 < s_2 < 0$, and the oscillatory branch terminates in a heteroclinic orbit connecting the two saddle-points¹³. For larger r_s , the small negative eigenvalue s_2 decreases along the branch until $s_2 = s_3$; the two eigenvalues

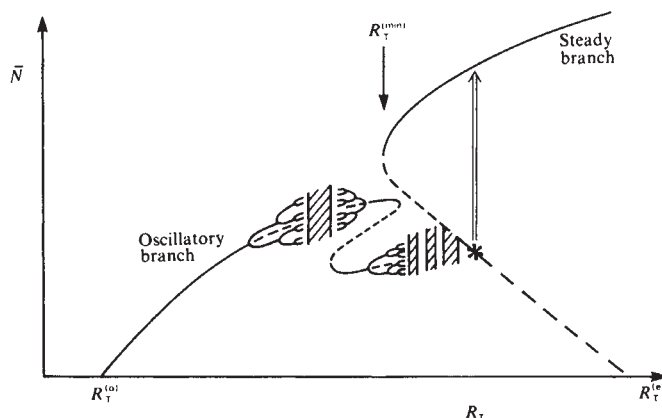


Fig. 3 Variation of a measure of convective transport, $\bar{N}$, with R_T along the branches of oscillatory and steady solutions for equations (1)–(3) with $R_S = 10^4$, $\tau = 10^{-1/2}$ and $\sigma = 1$. Solid and broken lines denote stable and unstable solutions respectively. Some of the bifurcations are shown and chaotic behaviour is realized in the shaded regions. Proceeding from $R_T^{(o)}$ along the oscillatory branch we find the bubble of bifurcations on the first part of the branch, followed by a pair of bifurcations leading to the second part. Only one more bubble, with its periodic windows, is shown explicitly and the final heteroclinic bifurcation is indicated by an asterisk.

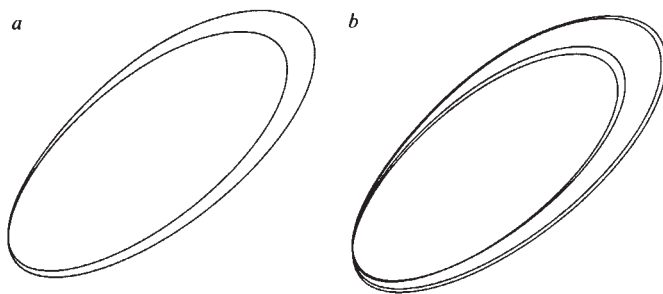


Fig. 4 Period-doubling for the fifth-order system with $\omega = \frac{8}{3}$, $\sigma = 1$, $\tau = 10^{-1/2}$ and $r_s = 15$. Limit cycles in the c - e plane for *a*, $r_T = 13.5$ or $R_T = 8,876$ (asymmetrical) and *b*, $r_T = 13.56$ or $R_T = 8,916$ (period 2). Subsequent period doublings lead to chaos by $r_T = 13.5635$ or $R_T = 8,918$.

then merge to form a complex conjugate pair, so that the heteroclinic orbit spirals into two saddle-foci. With $r_s = 15$ the first three eigenvalues satisfy Shil'nikov's inequality (11) for $38.65 > r_T > 10.22$. Note that this condition cannot be satisfied when r_s is too small since $s_1 = 0$ at $r_T^{(\min)}$ (ref. 12). Moreover, although the model is five-dimensional, the last two eigenvalues s_4 and s_5 are sufficiently contracting that the system is effectively three-dimensional and the Shil'nikov mechanism is unchanged. This suggests that chaos appears as the result of heteroclinic explosions. Similar, but not identical, behaviour is found in the Lorenz equations with $\omega = \frac{1}{4}$, $\sigma = 10$ (ref. 19).

The Shil'nikov mechanism has been shown to be present in another system of partial differential equations²³. Although the full system (1)–(5) is infinite-dimensional, we conjecture that all but the first three eigenvalues are sufficiently large and negative for the dynamics to remain effectively three-dimensional in the neighbourhood of the unstable steady branch. Moreover, the full system contains a heteroclinic orbit¹³; hence we expect that the Shil'nikov mechanism is responsible for heteroclinic explosions in equations (1)–(3) as well.

Periodic windows

As R_T is increased, symmetrical oscillations of the type shown in Fig. 2*f* persist in the partial differential equations until

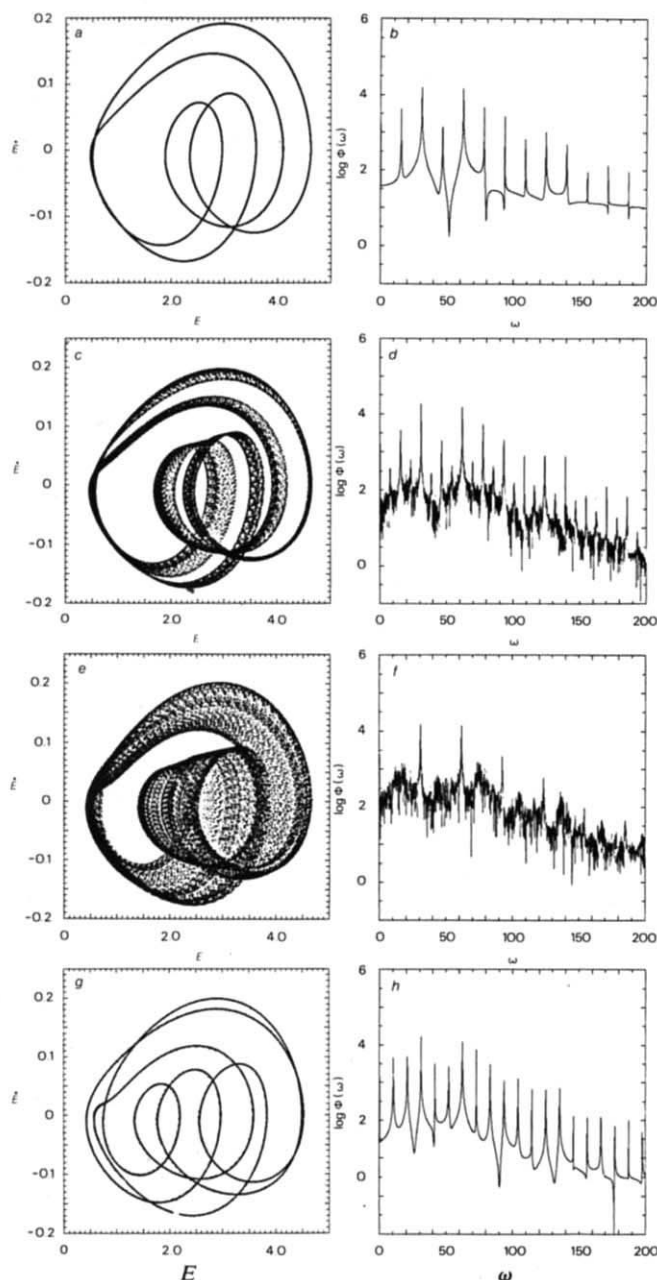


Fig. 5 Solutions on the second oscillatory branch, with $\lambda = 1.5$, for the partial differential equations. Trajectories in the kinetic energy phase plane (kinetic energy, E , plotted against its time derivative, $\dot{E}$) and the semilogarithmic plots of the kinetic energy power spectrum $\Phi(\omega)$, as a function of frequency, ω , for *a*, *b*, $R_T = 10,450$ (asymmetrical); *c*, *d*, $R_T = 10,475$ (semiperiodic); *e*, *f*, $R_T = 10,500$ (semiperiodic) and *g*, *h*, $R_T = 10,510$ (period 3, symmetrical). Orbits are described in a clockwise sense.

$R_T \approx 10,500$, when they lose stability. Solutions are then attracted to a second oscillatory branch which, for $\lambda = 1.5$, appears when $R_T \approx 10,300$; the hysteresis suggests that the two branches are connected by a pair of saddle-node bifurcations. We have investigated the behaviour of solutions along the whole oscillatory branch by increasing R_T and using nearby solutions for initial conditions. The relationship between the two branches and the bifurcation patterns that were found are shown schematically in Fig. 3. There may in fact be many branches but we were only able to identify these two. At the beginning of the second branch the oscillations are symmetrical with period 1 but of different form from those on the first branch. Subsequent bifurcations lead to asymmetry and another period-doubling cascade. Figure 5*a* shows a limit cycle for an asymmetrical solution with period 1, projected onto the kinetic energy phase plane. The corresponding power spectrum, $\Phi(\omega)$,

where ω is the frequency, is illustrated in Fig. 5*b*: there are sharp peaks at the basic frequency $\omega_0 = 4\pi/P \approx 30$ (where P is the full period of the basic cycle) and at $\frac{1}{2}\omega_0 \approx 15$. Note that the detailed appearance of the power spectra may be sensitive to changes in the interval T for which solutions are obtained, since the ratio T/P is not generally an integer. Beyond the accumulation point of the period-doubling sequence semiperiodic trajectories²⁴ are found, like that in Fig. 5*c*. The corresponding spectrum is noisy but with peaks clearly visible at frequencies ω_0 , $\frac{1}{2}\omega_0$, $\frac{1}{4}\omega_0$, $\frac{1}{8}\omega_0$ and combinations of those values. As R_T increases, so does the noise, and the peaks are gradually submerged: Fig. 5*e* and *f* show chaotic behaviour with only peaks at ω_0 and its harmonics visible in the spectrum.

Within the range of values of R_T that yields aperiodic solutions on the second oscillatory branch, there are various narrow windows with periodic solutions. The most prominent of these exhibit symmetrical limit cycles with period 3. Figure 5*g* shows such a solution for $R_T = 10,510$, with the corresponding sharply peaked spectrum in Fig. 5*h*. As R_T is increased, the appearance of this solution is preceded by intermittency² and is followed by bifurcations to asymmetry and then to period 6, and so on, until chaos reasserts itself. Eventually there is an inverse cascade of period-halving bifurcations leading to solutions with period 1, followed by another transition back into chaos through a period-doubling sequence as R_T is increased. Although solutions are typically aperiodic, further periodic windows can be found and we have located two more with period 3. Such behaviour appears to be characteristic of the dynamics associated with the Shil'nikov mechanism and detailed investigation of a fifth-order model of magnetoconvection has revealed an intricate sequence of period-doubling windows interspersed with aperiodic bands²². The windows can be ordered to provide a natural sequence that ends with one based on a symmetrical limit cycle of period 3. As with thermosolutal convection¹⁸, the actual sequence of bifurcations has a bubble structure, produced by a double-valued passage through the natural sequence and back again as R_T is increased. We have seen that a similar bubble structure is present on the first oscillatory branch, though no subsidiary windows were detected. We have identified at least two complete bubbles on the second branch, with indications that there are more. Possibly there are infinitely many bubbles, culminating in a heteroclinic orbit, as in the Lorenz equations¹⁹. Note that a second oscillatory branch can be found for the truncated system (6)–(10) as well, though for parameter values that are distinctly different from those considered here.

For $R_T^{(min)} < R_T < R_T^{(e)}$ there are two steady solution branches, of which the upper is stable and the lower unstable. The second oscillatory branch terminates when $R_T \approx 11,060$ (for $\lambda = 1.5$). For $R_T \geq 11,065$ all trajectories are attracted to the stable fixed point on the upper steady branch. Towards the end of the oscillatory branch solutions become more strikingly chaotic. Figure 6 shows results for $R_T = 10,625$ and $R_T = 11,000$. These values yield aperiodic solutions but belong to different bubbles: the trajectories fill much more of phase space than those in Fig. 5 and peaks have been eliminated from the spectra. Near the end of the oscillatory branch the basic cycle tends to be interrupted while solutions hover in the neighbourhood of the unstable steady solution; in phase space, trajectories spiral slowly in towards the unstable fixed points before escaping rapidly from their neighbourhoods. This is consistent with the existence of a heteroclinic connection between fixed points with eigenvalues that satisfy Shil'nikov's inequality (11).

Conclusions

It is well known that dissipative systems of difference or ordinary differential equations can exhibit period-doubling bifurcations, leading to chaos, interrupted by periodic windows¹. We have shown here for the first time that this route to chaos² can be found for partial differential equations too. In addition, we have demonstrated how truncated model systems, derived from physical intuition or by asymptotic methods, can be used to interpret the dynamics of more complicated configurations. However,

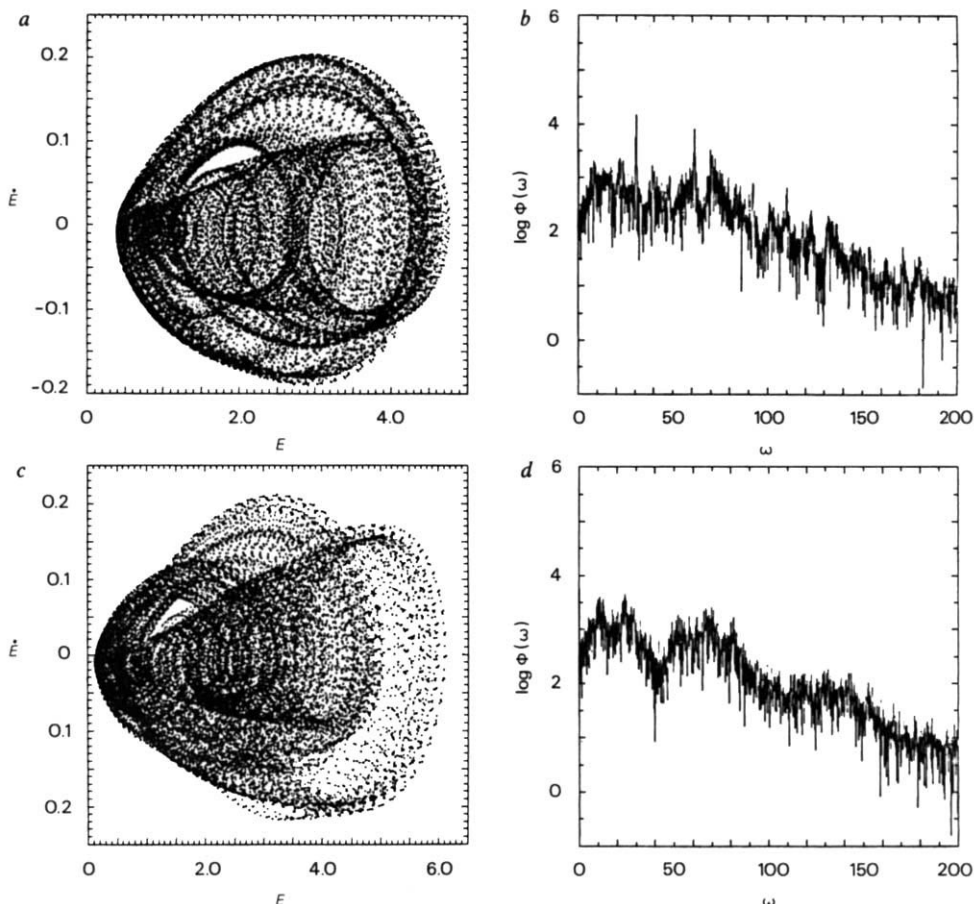


Fig. 6 Chaotic behaviour on the second oscillatory branch. As Fig. 5 but with *a*, *b*, $R_T = 10,625$ and *c*, *d*, $R_T = 11,000$. Comparison with Fig. 5 will help in interpreting these trajectories.

the robustness of the attractor that we have isolated requires further study, for it is not yet clear how far the behaviour outlined above will persist if the constraints of symmetry and two-dimensionality are relaxed.

Previous studies of partial differential equations have included a proof of the existence of horseshoes in reaction-diffusion equations²³ and for magnetoelasticity²⁵, while other computations have revealed a quasiperiodic route to chaos²⁶⁻³⁰. The presence of period-doubling sequences, with examples of periodic windows, bubbles and hysteresis, has been established experimentally for circuits³¹⁻³³, for lasers³⁴ and for the chemical Belousov-Zhabotinsky reaction^{35,36}. More important, successive period-doubling sequences have been unambiguously observed in experiments on Rayleigh-Bénard convection in liquid helium³⁷, mercury^{38,39} and water⁴⁰. Though its realization may require small aspect ratios or other lateral constraints, the rich behaviour that we have described appears to be a property of real fluid systems.

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Seismic reflections from subducting lithosphere?

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Data from two short reflection lines obtained using explosives and an airgun source, together with offshore and onshore refraction profiles and a gravity interpretation, provide evidence of coherent reflections between the subducting oceanic Juan de Fuca and overriding continental America plates.

To study the structure of the transition between the subducting oceanic Juan de Fuca and overriding continental America plates, the Vancouver Island Seismic Project (VISP) was conducted in 1980 by the Canadian Consortium for Crustal Reconnaissance using Seismic Techniques (COCRUST). Offshore and onshore refraction profiles comprised the major portion of the programme, but two short reflection lines were also run to test the feasibility of obtaining coherent reflections to upper mantle depths in this complex geotectonic environment, and of acquiring deep reflections in coastal areas using an airgun source. Correlation of the observed reflectors from explosive and airgun sources with geological boundaries is hindered by the 10-km explosion profile length; however, reflectors at 4.4 and 7.0 s correspond in depth to a midcrustal refractor in the overriding continent and to the top of the subducting lithosphere, as interpreted from previous gravity modelling¹ and preliminary offshore-onshore VISP refraction results². The deeper and tentatively identified reflections would be within the subducting lithosphere.

Objectives

Keen and Hyndman³ have reviewed our knowledge of this complex tectonic regime where the Explorer and Juan de Fuca Plates are being subducted at rates of 2–3 cm yr⁻¹ beneath the America Plate. Figure 1 outlines the plate boundaries and the direction and relative speeds of plate motions while Fig. 2 presents the pre-VISP structural model of Riddihough¹ along GR (Fig. 1). This model is based primarily on gravity data but uses both controlled source and earthquake seismic evidence where available; it is also constrained by the essential features of an active subduction zone. On a local scale, the site of the reflection profile (RL in Fig. 1) is also complex. The major portion of the 10-km line is along or close to a major fault which separates the Jurassic Island intrusions and Upper Triassic Quatsino limestones on the north from Lower Jurassic Bonanza volcanics to the south. With the limited line length and structural complexity, the objectives of the reflection experiments were principally to test the feasibility of the tech-

niques in this environment rather than to provide geological interpretations. However, the gravity and preliminary VISP refraction results^{2,4,5} do allow tentative correlations to be made.

Profiles

The 1,200% coverage on the explosion reflection line was achieved using shot hole and station spacings of 200 and 100 m

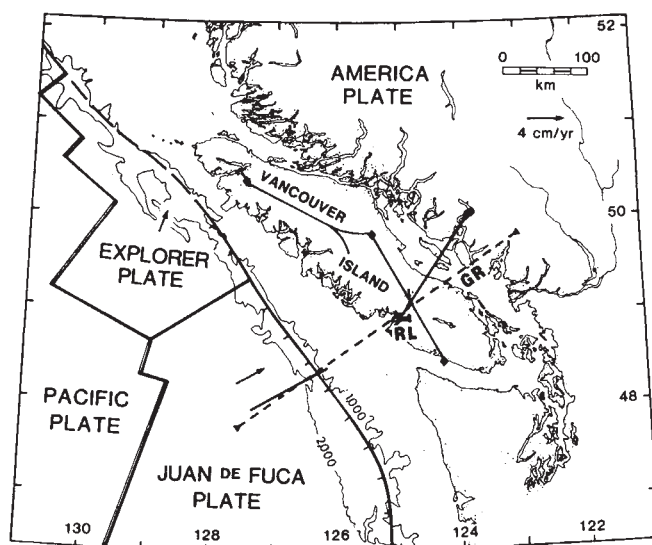


Fig. 1 Tectonic map of the western Canadian continental margin and location map for the VISP program. Arrows show direction and speed of plate motions relative to the America plate. Solid lines onshore are locations of refraction profiles^{2,4}; diamonds are shot points. Off the shelf, the solid line is the location of the explosion profile². The dashed line (GR) is the gravity model cross-section shown in Fig. 2. RL is the location of the reflection profiles of Figs 3 and 4.

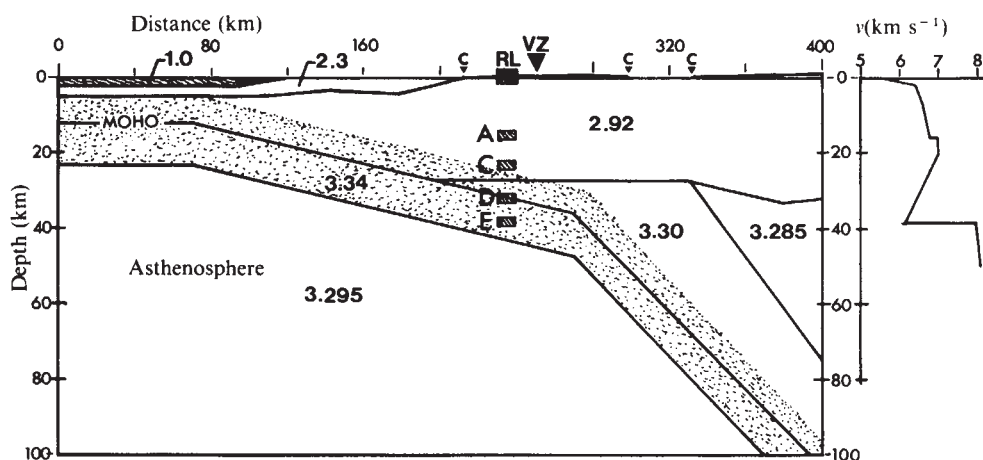


Fig. 2 Gravity model cross-section of Riddihough¹ across southern Vancouver Island; densities are in g cm⁻³. Shaded area is the subducting oceanic lithosphere. Coast lines are indicated by an arrow labelled C. RL identifies the location of the reflection profile; equivalent depths for reflectors A, C, D and E of Fig. 3 are marked. The heavy arrow with VZ shows the approximate location of the intersecting refraction line along the island from which the velocity-depth curve to the right of the cross-section was derived⁴. Vertical exaggeration, 2:1.

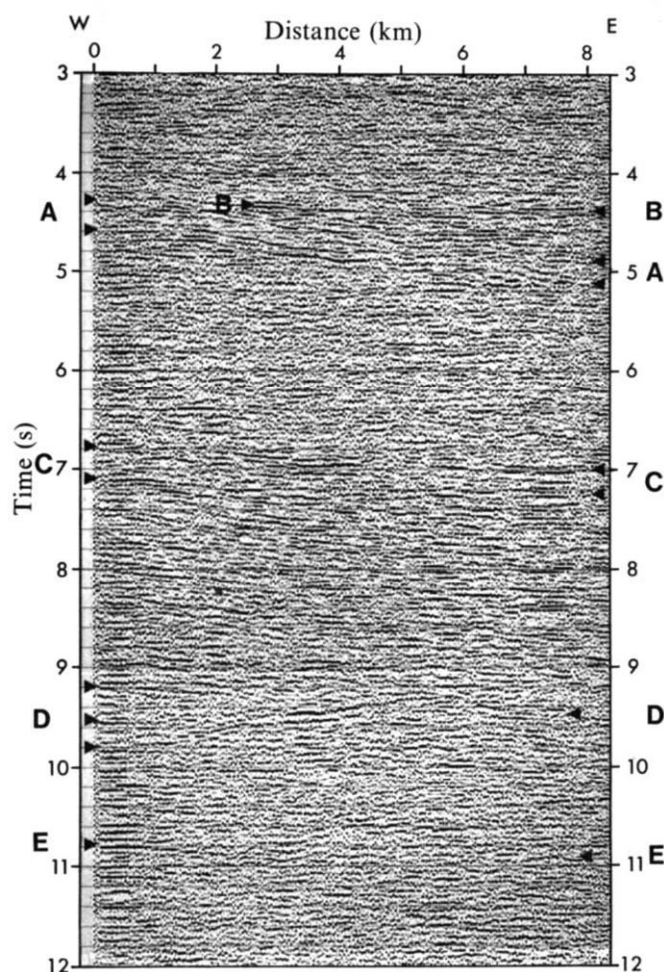


Fig. 3 Processed record section from the 1,200% common reflection point explosion profile. Reflections discussed in the text are identified with symbols and labelled A to E. D and E are more speculative than the shallower events.

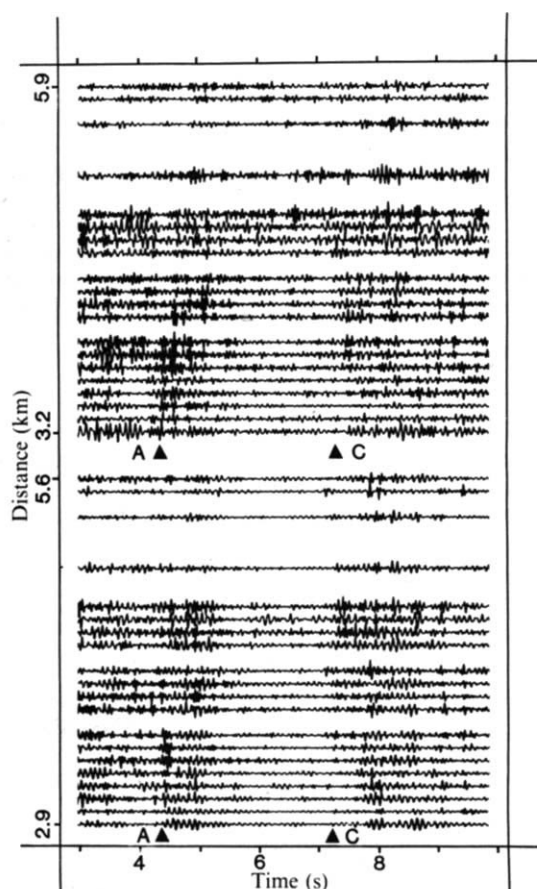


Fig. 4 Uncorrected seismic sections recorded on land reflection system from a 32-l airgun source fired offshore. Coherent energy is present near 4.4 and 7.2 s, times which correspond to the upper reflectors (A–B, C) on the explosion profile (Fig. 3).

respectively. A 20-s record sampled at 4 ms was processed with normal moveout (NMO) corrections based on a smoothed version of the refraction velocity model (Fig. 2). In the airgun programme, the 48-channel seismic system was held fixed and the 32-l airgun fired approximately every 200 m over distances of 0.5 to 10 km along a profile in an adjacent inlet. The profile was repeated to provide six-fold redundancy. Figure 3 shows the corrected and stacked explosion section to which automatic gain control, crooked line processing, bandpass filtering (8–30 Hz) and a coherency filter have been applied. Figure 4 shows two uncorrected, single-shot airgun records which have been bandpass filtered (8–30 Hz), gain controlled and from which noisy channels have been deleted. On neither profile was coherent energy observed at times < 4 s. On the explosion section reflections are present near 4.4, 7.0, 9.5 and 10.8 s while on the airgun profile only the two shallow reflections are evident. Unfortunately trace-coherent noise bursts, present on most airgun records, preclude stacking and filtering procedures which could enhance these reflections.

Reflection times can be converted to approximate depths using a travel time–depth curve constructed from the velocity model of Fig. 2. The reflector depths at the western end of RL correspond to 14, 23, 31, and 37 km. Thus in this complex environment good reflections have been obtained to depths which are characteristic of the lower oceanic lithosphere or the continental Moho, while airgun penetration to mid-crustal (~23 km) depths has been achieved.

The upper reflector (A) is at ~14 km at the west and dips eastward at ~15° to a depth of 16 km. This is overlain by a flat-lying reflector (B), the combination perhaps indicating shal-

low thrust faulting or some other tectonic discontinuity, or an unconformity. These depths correspond closely to the 15.5-km depth of the discontinuous velocity increase on the refraction profile (Fig. 2). Correlation of this reflector with a geological boundary is difficult as estimates for total thickness of recognized lithological units under Vancouver Island are <10 km (refs 6, 7). The next coherent reflections (C) range in depth from about 23 to 25 km with an eastward dip of ~7°. While the simple gravity model shows no equivalent density boundary, the depths correspond closely to the top of the subducting oceanic plate, which dips at similar angles, in Riddihough's model (Fig. 2). More significantly, our continuing interpretation⁵ of the extensive VISP onshore-offshore refraction data, which is providing a more complex structural model, indicates that beneath the reflection line, the top of the subducting lithosphere is dipping eastward at ~5° at a depth of 22 km.

Below reflector C, to times of ~12 s, there exists a plethora of short, coherent reflection segments, some of which are likely to be true reflections, some possibly multiples and others artefacts of the processing sequence. Any true reflectors would be located within the lower crustal or upper mantle sections of the descending lithosphere. Two relatively coherent phases (D and E) are marked. The shallower reflection (31–33 km) corresponds to the depth of the oceanic Moho in the descending lithosphere model. The deeper (~38 km) reflection may also represent internal structure within the plate. Note that this depth also corresponds to the base of the continental crust in the refraction model (Fig. 2). Accommodation of this interpretation would require major changes in the simple subduction model. The significant result from this short line is that coherent

reflected energy is obtained from depths to ~40 km in a complex geotectonic environment.

Conclusions

A 10-km 1,200% deep reflection profile at a convergent margin shows two clear reflectors, at depths near 16 and 24 km, which correspond to a reflector within the overriding crust and the top of the subducting lithosphere. These reflections are also observed on records using an airgun source. Two weaker events corresponding to depths within the subducting lithosphere have also been tentatively identified. We conclude that reflection profiling using explosives will be a useful, and necessary, interpretative technique at convergent margins and that the use of

large airguns to obtain deep seismic reflections warrants further investigation.

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Expression of HLA-DR antigens at the surface of mouse L cells co-transfected with cloned human genes

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The H-2 I region in the mouse and the HLA-D region in humans contain a set of polymorphic genes which encode Ia antigens and control the immune response. Cloned genes for the different polypeptide chains of one of the human Ia antigens, HLA-DR, have been used for the co-transformation of mouse L cells. Expression of HLA-DR antigens at the surface of transfected mouse cells has been documented with monoclonal antibodies.

A SET of genes within the major histocompatibility complex (MHC) controls essential aspects of the immune response such as intercellular recognition and cooperation, alloreactivity and the immune responsiveness to certain antigens^{1–3}. These genes map in the I region of the murine MHC (I-A and I-E subregions) and in the HLA-D region of the human MHC (Fig. 1a). They code for cell-surface glycoproteins, referred to as Ia antigens or class II antigens. They are characterized by a high degree of polymorphism and are expressed primarily on B lymphocytes and antigen-presenting cells such as macrophages^{4–6}. Human class II antigens, like those of the mouse, are dimeric molecules consisting of two transmembrane polypeptides, the α -chain and the β -chain, the latter being clearly responsible for the polymorphism in the case of HLA-DR⁷. In addition, a third chain, called invariant (In), is found intracellularly associated with the mouse and human class II molecules^{8,9}. It is believed to be involved in the assembly and transport of the Ia antigens^{10,11}.

At least three distinct types of class II antigens have been identified in man, all encoded in the HLA-D region and presumably in distinct subregions: the HLA-DR antigens^{4–7}, and the more recently described DC¹² and SB antigens¹³. These subregions are centromeric to the loci coding for HLA-A, B, C class I antigens (Fig. 1a). They are all characterized by serological and cellular alloreactivity. HLA-DR displays the most extensive polymorphism. We have studied this system at the molecular level and have first isolated cDNA clones for the polymorphic β -chain of HLA-DR and HLA-DC¹⁴, as well as cDNA clones for the non-polymorphic DR α -chain¹⁵ and for the DR-associated invariant chain (in preparation). These clones have been used to identify the corresponding genes from human genomic libraries¹⁶. Other laboratories have reported the isolation of cDNA clones for HLA-DR and -DC α -chains^{17,18} and for DC β -chains¹⁹. The nucleotide sequence of one of our full-length

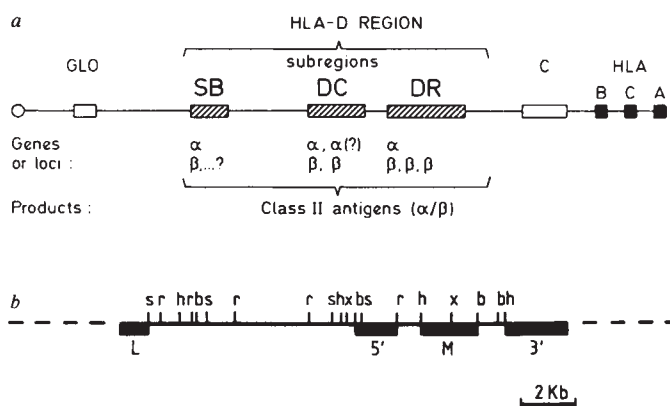


Fig. 1 a, Schematic representation of the human MHC. Evidence for the individual genes shown in the HLA-D region, and in particular for the existence of multiple DR β -chain genes and DC β -chain genes, has been discussed elsewhere^{16,20,23}. The relative position of DC and DR is indicated on the basis of preliminary hybridization experiments performed with deletion mutants in collaboration with D. Pious (in preparation). The HLA-A, B, C loci encode the heavy chain of class I antigens. C Represents genes involved in the complement system. b, Restriction map of the λ phage recombinant λ DR β -10 containing an HLA-DR β -chain gene (provided by J. Gorski and P. Rollini). This clone was isolated from a genomic library made from DNA of an HLA-DR 4, w6 B-cell line. Its identification as DR w6 and the analysis of other clones for other HLA-DR β -chain genes are described elsewhere¹⁶. The following restriction endonucleases are used: b, *Bam*HI; h, *Hind*III; r, *Eco*RI; s, *Sac*I; x, *Sba*I. Black boxes indicate DNA segments hybridizing to ³²P probes derived from a DR β -chain cDNA clone¹⁴ and specific for the leader (L), 5', middle (M) and 3' portions of the gene, respectively.

Table 1 Transformation of mouse Ltk⁻ cells with HLA-DR genomic clones

Expt	Genomic clones				Amount of Ltk ⁺ DNA carrier	No. of tk ⁺ wells	Total no. of tk ⁺ colonies
	Name	Gene	Amount	Enzymatic treatment			
1	λ DR β-10	β DR	1 μg	<i>Nru</i>	19 μg	9/9	18
	pAGO	<i>tk</i>	50 ng	<i>HindIII</i>			
22	λ DR β-10	β DR	1 μg	<i>Nru</i>	14 μg	9/9	> 50
	λ DR α-H6A	α DR	1 μg	—			
	cos In (1,9,20)	{ In <i>tk</i>	{ 3 μg	<i>SalI</i>			

The thymidine kinase-deficient mouse L-cell line, Ltk⁻, (provided by P. Goldstein) was maintained in monolayer cultures in Dulbecco's modified Eagle medium (DME) supplemented with heated 10% fetal calf serum (FCS), 1 mM sodium pyruvate, 1% streptomycin, penicillin. Approximately 3×10^6 Ltk⁻ cells, in 25-cm² tissue culture flasks, were transfected as described elsewhere³⁵ with some modifications. Cells were incubated for 7 h at 37°C with calcium phosphate DNA precipitates and trypsin-dispersed into 9 wells of a 24-well tissue culture microplate (Costar). 24 h later, cultures were switched to HAT selection medium and grown further. Positive colonies were scored after 3–4 weeks and tk positive (tk⁺) co-transformants from each well were maintained in 25-cm² tissue culture flasks and passaged as mixed populations of tk⁺ co-transformants (1₁ to 1₉ and 22₁ to 22₉). The following DNAs were used as indicated: (1) λ bacteriophage λ DR β-10, which contains an HLA-DR β-chain gene (see Fig. 1b). (2) λ phage λ DR α-H6A which contains the HLA-DR α-chain gene³⁵ (provided by A. J. Korman and J. L. Strominger). (3) Cosmid recombinants In 1, 9 and 20, which contain the invariant-chain gene (in preparation) in the cosmid vector pOPF 1³². These recombinants will be described elsewhere (in preparation). The cosmid vector also contains the HSV *tk* gene. (4) Plasmid pAGO³⁴, which contains the HSV *tk* gene. Before performing co-transfection experiments with all the HLA-DR genes together, we established that each of these genes was expressed in the transfected mouse cells. This was done by analysis by Northern blot hybridization (data not shown) and indicated that the α-, β- and invariant-chain genes used are functional.

HLA-DR β-chain cDNA, and its corresponding amino acid sequence has been published²⁰. Cross-hybridization studies between human and mouse class II genes and structural comparisons indicate that the HLA-DR complex is related to mouse I-E and HLA-DC to mouse I-A^{21–23}. More recently, we have established that at least three different loci exist for HLA-DR or DR-like β-chains^{16,23}, all distinct from those of DC and SB β-chains (Fig. 1a). This implies that, in addition to the existence of β-chain genes for the structurally distinct HLA-DR, -DC and -SB Ia antigens, the HLA-DR subregion itself is genetically complex. It is essential in the study of the Ia system to understand the relative contribution and respective role of individual D region genes in the known serological specificities, and in the known cellular reactions, studied as alloreactivity and as MHC-restricted recognition.

Genes for cotransformation

A correlation between a given gene and its phenotypic expression, analysed either serologically or functionally, would require the expression of cloned genes and the recognition of their products on transfected cells. DNA-mediated gene transfer into mouse L cells²⁴ with a single gene (in addition to a selection marker) has been achieved with the stable expression of its protein product²⁵. In the case of transfection with class I MHC genes, the product of a single gene (HLA or H-2 heavy-

chain) associates with a second chain, β₂-microglobulin, already present in the transformed cell^{26–28}. To transform cells for the expression of human HLA-DR multimeric molecules, we attempted the co-transformation of mouse L cells with four different genes: the genes for the polymorphic DR β-chain, the DR α-chain the DR-associated invariant (In) chain and, in addition, the gene for herpes simplex virus (HSV) thymidine kinase (*tk*), which provided a selection marker (Table 1). Previous studies have shown that the *tk* gene need not be covalently linked to another gene for the co-expression of both genes²⁹. In our experiments, the different genomic clones used were linearized with restriction endonucleases but were not ligated.

In experiment 1 (Table 1), two genomic clones were used: an HLA-DR β-chain gene¹⁶ from a DR 4, w6 DNA library in λ Charon 30 vector (λ DR β-10, Fig. 1b) and the plasmid pAGO which provides the *tk* gene as a selective marker³⁰. In experiment 22 (Table 1), the λ DR β-10 recombinant was used with the HLA-DR α-chain gene³¹, also in λ bacteriophage (λ DR α-H6A) and the gene for the human invariant chain (in preparation) in the cosmid vector pOPF1³². This vector also provides the *tk* gene as a selection marker. As expected, tk⁺ transformants were obtained in all cases following selection in hypoxanthine-aminopterin-thymidine (HAT) medium (Table 1). The transformation efficiency differed in the two experiments shown, ranging from 18 to more than 50 colonies for

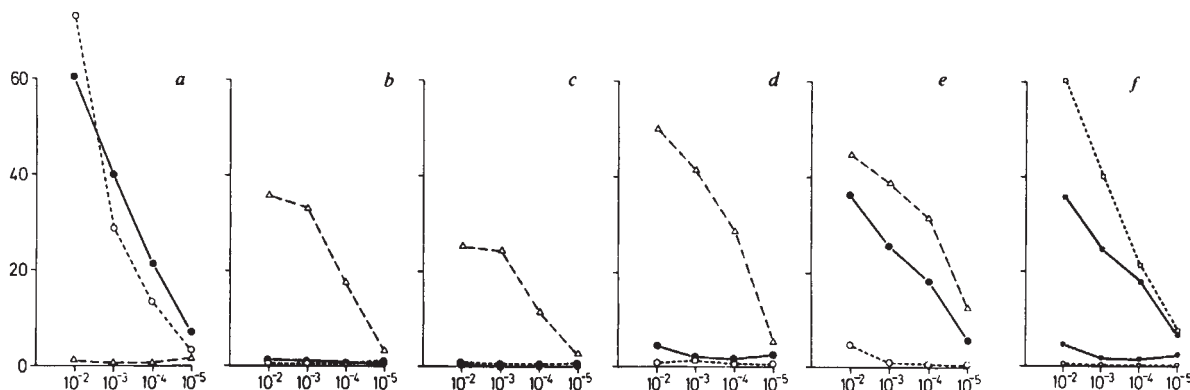


Fig. 2 Analysis of co-transformants by a cell binding assay. The experimental conditions are as described in Table 2 legend. Abscissa, dilution of monoclonal antibodies; ordinate, ¹²⁵I protein A-bound (c.p.m. $\times 10^{-3}$). a, Human B-cell line DR 4, w6; b, mouse Ltk⁻ cells; c, co-transformant 14; d, co-transformant 22; e, co-transformant 22. Monoclonal antibodies used in panels a–e: ○, anti-HLA-A, B, C; ●, pool of anti-HLA-DR; △, anti-H-2K^k. f, Binding of anti-HLA-DR monoclonal antibodies (Table 2) with the following cell lines: □, human B-cell line DR 4, w6; ■, co-transformant 22; ●, co-transformant 22; ○, mouse Ltk⁻ cells.

Table 2 Cell-surface binding assay of co-transformants

Antibody		Expt	Cell lines											
Specificity	Dilution		HLA-DR (4, w6)	Ltk ⁻	1 ₄	22 ₁	22 ₂	22 ₃	22 ₄	22 ₅	22 ₆	22 ₇	22 ₈	22 ₉
HLA-A, B, C*	10 ⁻²	1	44,497	213	1,209	833						5,999		4,878
HLA-A, B, C*	10 ⁻²	2	73,068	559	239		572							4,405
HLA-A, B, C*	10 ⁻²	3	35,679	1,945				1,395	616	825	593	1,308		
Control ascite	10 ⁻²	4	879	475	734				1,398				396	638
Normal mouse serum	10 ⁻²	5	286	3,264		5,317					6,787	2,033		
H-2K ^k *	10 ⁻³	1	1,306	29,856	28,192	45,936						47,862		39,934
	10 ⁻²	2	1,247	35,655	25,055		49,603							43,962
	10 ⁻²	3	3,075	48,115				23,602	15,970	26,075	27,852	46,078		
	5 × 10 ⁻³	4	3,037	63,917	43,456				54,319				66,819	57,379
	10 ⁻²	5	787		22,150	20,196					24,070	20,032		
HLA-DR	10 ⁻²	1	47,389	431	391	5,832						6,680		34,646
	10 ⁻²	2	60,284	1,240	421		4,292							35,517
	10 ⁻³	3	55,875	1,916				6,476	1,643	1,293	1,091	5,340		
	10 ⁻²	4	72,822	3,175	1,303				2,414				5,288	55,328
	10 ⁻²	5	30,096		874	7,051					4,935	6,899		

Transformed and control cells were assayed as follows. Cells were distributed in V-bottomed microplates (Dynatech) at 5×10^5 cells per well, centrifuged at 4 °C and resuspended in 25 μ l of DME-2% FCS containing antibody at the indicated dilution. After a 2-h incubation at 4 °C with gentle shaking, cells were washed three times with DME-2% FCS and resuspended in 25 μ l of DME-2% FCS containing ~ 3.5 ng (10^5 c.p.m.) of 125 I-protein A (Amersham). Finally, after four washes in DME-2% FCS, cell-associated radioactivity was measured in a γ counter. Cells tested include tk⁺ co-transformants from well 1₄ (Table 1) and tk⁺ co-transformants from wells 22₁ to 22₉ (Table 1). Untransformed Ltk⁻ cells (H-2^k) and a human lymphoblastoid cell line (HLA-DR 4, w6) were used as control cells. The following antibodies were used: (1) B9.12.1 anti-HLA-A, B, C monoclonal antibody³⁶ (protein A-purified) which recognizes a monomorphic determinant on human class I heavy chains. (2) Control mouse ascites fluid. (3) Normal mouse serum. (4) 11.4.1 anti-H-2K^k monoclonal antibody³⁷ (protein A-purified), which recognizes murine class I antigens. (5) D1-12, D4-22 and BT 2.2 anti-HLA-DR monoclonal antibodies (ascites fluid) used as a mixture. These three monoclonal antibodies recognize common determinants of HLA-DR β -chains shared by all DR specificities and the reactivity of these antibodies has been described in detail elsewhere^{33,34}. All antibody preparations were centrifuged for 15 min at 10,000 r.p.m. at 4 °C before use. * Starting material for HLA-A, B, C and H-2K^k antibodies was at 10 mg ml⁻¹.

the 9 wells (Table 1). Co-transformants from individual wells were amplified for further study. Each of these cultures represents the progeny of several colonies and are referred to as 1₁ to 1₉ and 22₁ to 22₉.

HLA-DR expression

In the case of transformation for the expression of Ia antigens such as HLA-DR, the four different genes used in the co-transformation must be expressed, the relevant polypeptide products must assemble correctly intracellularly, and the HLA-DR complex must appear on the cell surface with the correct antigenic specificity. The analysis of the tk⁺ co-transformants at the mRNA level (Northern blots) showed that most of them expressed the human HLA-DR genes (data not shown). We then analysed the tk⁺ co-transformed cells for the presence of cell-surface HLA-DR antigens, using three HLA-DR-specific monoclonal antibodies^{33,34}. Three procedures were used: a cellular binding radioimmunoassay (Table 2, Fig. 2), immunofluorescence analysis (Fig. 3) and immunoprecipitation of iodinated cell-surface proteins followed by polyacrylamide gel analysis (Fig. 4). In all cases controls were included, involving control cells (such as Ltk⁻, Ltk⁺ co-transfected with the DR β -chain gene alone (1₄) and a human lymphoblastoid cell line HLA-DR 4, w6 and control antibodies (such as anti-HLA-A, B, C and anti-H-2K^k).

Table 2 gives the results of several binding assays with co-transformants obtained from experiment 1 (well 1₄) and experiment 22 (wells 22₁-22₉) described in Table 1. All the mouse cell lines, whether transformed or not, react with anti-H-2K^k monoclonal antibodies. The human lymphoblastoid cell line (HLA-DR 4, w6), which had been used as the source of DNA for the genomic cloning, reacts with the anti-HLA-DR monoclonal antibody pool. Analysis of cells from the nine cultures derived from the individual wells of experiment 22 (transfection with DR α -, β - and In-chain genes) showed that some lines (22₄, 22₅, 22₆) do not react with anti-DR monoclonal antibodies, that some are unequivocally DR-positive (22₁, 22₂, 22₃, 22₇ and 22₈) although considerably less reactive than the human cell control, and that one line (22₉) reacts very strongly with anti-DR antibodies and compares well with the control human lymphoblastoid cell. By contrast, untransformed Ltk⁻ cells, and

co-transformant 1₄ (from transfection with the DR β -chain gene only) are negative. Table 2 also shows that similar results are obtained with these cell lines on repeated assays.

The same binding assays were performed with different concentrations of the various antibodies, including those used as positive and negative controls. Figure 2a-e shows the dilution curves obtained in control cell lines (a-c) and in a weak (d) and a strong (e) DR⁺ co-transformant, 22₇ and 22₉, respectively. Figure 2f, which summarizes the dilution curves obtained with anti-DR monoclonal antibodies for the four cell lines tested, shows clearly that transformant 22₉ is reacting to DR antibodies in the same order of magnitude as the control B-cell line.

We have confirmed the expression of HLA-DR antigens on transformed mouse cells by indirect immunofluorescence analysis. Figure 3 shows the results which involved a control human B-Cell line (a, d), untransformed mouse L cells (b, e) and co-transformed cells 22₉ (c, f). The co-transformant 22₉ is clearly stained with anti-HLA-DR monoclonal antibodies, almost to the same extent as the human B-cell line. In contrast, the untransformed mouse L cells are totally negative. Figure 3c and f compares the phase-contrast picture (top) with the immunofluorescence (bottom) for two cells which do not label at all and four brightly stained cells. Because, as indicated above, the co-transformants were propagated and analysed as mixtures of tk⁺ clones originating from each well, one would expect different clones in each culture. It is likely therefore that culture 22₉ consists of a mixture of DR⁺ and DR⁻ cells; in this experiment, the tk⁺ DR⁻ cells provide a rigorous internal control for nonspecific reactivity. In addition, the presence of both DR⁻ and DR⁺ cells in a given culture suggests that the weaker reactivity of some co-transformants (see Table 2) might be due to the presence of several DR⁻ clones together with a strongly reactive DR⁺ clone.

Molecules recognized as HLA-DR antigens on the surface of the transformed mouse cells were also analysed by polyacrylamide gel electrophoresis. Cell-surface proteins were labelled with 125 I and the cells were reacted with anti-HLA-DR monoclonal antibodies before lysis. The antigen-antibody complex was bound to protein A-Sepharose, and the immunoabsorbed radioactive proteins were analysed on polyacrylamide gels. As can be seen in Fig. 4, the typical pattern of cell-surface HLA-

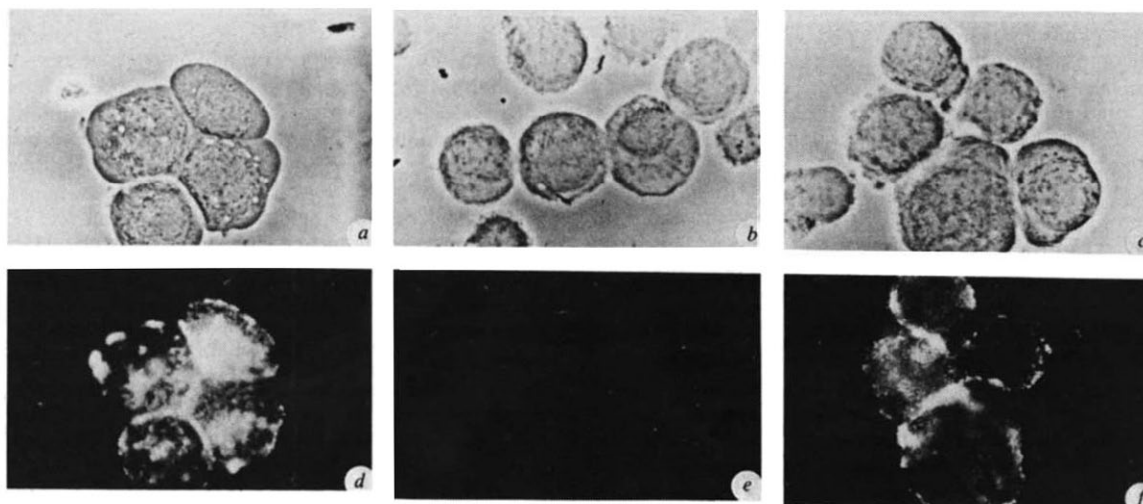


Fig. 3 Detection of cell-surface HLA-DR antigens on co-transformant cells by immunofluorescence. 5×10^5 cells were first incubated for 30 min in ice with $25 \mu\text{l}$ of 10^{-2} dilutions of monoclonal antibodies. After three washes in DME-10% FCS, cells were incubated for 30 min at 4°C with $5 \mu\text{l}$ rhodamine-labelled rabbit anti-mouse immunoglobulin antiserum (gift of P. F. Piguet), and washed three times with phosphate-buffered saline-bovine serum albumin (PBS-BSA 10%). A drop of the cell suspension was spread with a cytofuge on microscope slides, air dried, fixed with methanol, mounted in glycerol and examined with a Zeiss photomicroscope 11 equipped with an epifluorescent condenser RS 111. Top panels, phase contrast, bottom panels, immunofluorescence. *a, d*, Human lymphoblastoid line HLA-DR 4, w6; *b, e*, mouse Ltk⁻ cells; *c, f*, co-transformant 22g. Cells were tested with the pool of anti-DR monoclonal antibodies (this figure), and with monoclonal antibodies to HLA-A, B, C and H-2K^k (data not shown).

DR α - and β -chains is observed with the human B-cell line²⁸ (lane 1). The mouse DR⁺ co-transformant (22g) also gave two polypeptide chains (lane 2), one identical in mobility to the α -chain and one migrating slightly more slowly than the β -chain of the B-cell line. This slight difference in mobility was observed on two separate experiments. It could reflect a difference in the glycosylation pattern, which has not prevented the recognition of the molecule as HLA-DR antigen. In this experiment, the overall cell-surface iodination of the adherent mouse cells was less efficient than the labelling of the human B cells grown in suspension. This experiment cannot therefore be interpreted quantitatively. It shows that the molecule responsible for the HLA-DR reactivity in the mouse transformed cells is composed of two chains very similar to those of the α/β HLA-DR molecule. With the same anti-HLA-DR antibodies, no polypeptide chain is precipitated in the case of mouse Ltk⁻ cells (lane 3) and co-transformant 1₄ (transfected with the DR β -chain gene alone (lane 4)).

Implications

The expression and recognition of HLA-DR antigens on the surface of transformed mouse cells implies that a series of events has taken place: penetration of the four genes used in the co-transformation; stable expression of the *tk* gene as well as the other genes coding for the various DR chains; synthesis of these different DR polypeptide chains; assembly of DR α/β dimers; and transport of these molecules as transmembrane proteins with the correct conformation recognized as HLA-DR by the monoclonal antibodies. The data presented here indicate that, indeed, co-transformed mouse cells express HLA-DR antigens on their surface. We believe this to be the first example of DNA-mediated gene transfer with the involvement of several genes necessary for the expression of a multimeric protein. It also represents the first formal identification of a gene for class II antigens by the analysis of the gene products. As three distinct HLA-DR β -chain genes have been identified in the human genome^{16,20,23}, it is of special interest that one of these genes alone encodes a product recognized as HLA-DR.

Mouse fibroblasts normally do not express their own class II genes. The relatively high level of expression of the transfected HLA-DR genes indicates that these human genes are not under

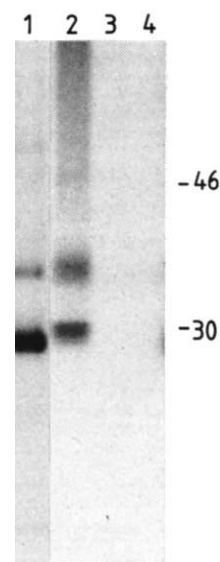


Fig. 4 Immunoprecipitation of ^{125}I -labelled protein with anti-HLA-DR monoclonal antibodies. 6×10^6 cells were iodinated with $150 \mu\text{Ci}$ of ^{125}I -iodine (3.7 GBq ml^{-1} ; Amersham) according to Hubbard and Cohn³⁸. Freshly iodinated cells were incubated with $400 \mu\text{l}$ of PBS containing $10 \mu\text{l}$ of anti-HLA-DR monoclonal antibodies (Table 2). After 2 h at 4°C , each sample was washed three times with cold PBS, lysed with 0.4 ml of buffer (150 mM NaCl, 1% deoxycholate, 1% Triton X-100, 0.1% SDS and 10 mM Tris-HCl, pH 7.8) sonicated for 15 s and spun for 15 min at 4°C in an Eppendorf centrifuge. The resulting cell lysates were subjected to immunoprecipitation as described previously³⁹. Radiolabelled immunoabsorbed molecules were eluted from the protein A-Sepharose CL-4B beads by boiling for 3 min in 10 mM Tris-HCl, pH 6.8, 15% glycerol, 2% SDS, 0.001% bromophenol blue, and an aliquot was loaded on a 12.5% polyacrylamide gel. Following electrophoresis, the gel was fixed, dried and exposed to preflashed Kodak X-AR films with intensifying screens for 24 h (track 1) and for 6 days (tracks 2, 3, 4). Lane 1, human B-cell line, lane 2, co-transformant 22g (see Table 1), lane 3, mouse Ltk⁻ cells; lane 4, co-transformant 1₄ (see Table 1). Molecular weight markers are shown ($\times 10^{-3}$).

the same control in the transformed mouse cells. Whether or not the human genes are integrated in a transcriptionally active region of the chromatin is unclear. The expression of HLA-DR genes by the transformed cells is a stable trait, has been observed in some cultures for over 4 months, and is a reproducible phenomenon. The different co-transformants which express various amounts of HLA-DR (see Table 2) show, by Northern gel analysis, a level of mRNA for the HLA-DR chains which correlates well with the level of DR expression (result not shown). One obvious interpretation for the different level of HLA-DR expression is the existence of mixed populations of clones in the individual cultures, with a consequently different ratio of DR⁺ and DR⁻ cells. Studies of cloned DR⁺ line will clarify this point. Because of the high stringency conditions used for the RNA/DNA hybridizations mentioned above, these RNA analyses also establish that the Ia-like mRNA expressed in the transfected HLA-DR⁺ mouse cells is of human and not of mouse origin. Transfection experiments with the HLA-DR α - and β -chain genes, but without the In-chain gene, indicate that the latter is not essential for the surface expression of HLA-DR antigen (in preparation). The precise role of the invariant chain gene in HLA-DR biosynthesis is under investigation with the co-transformation system described here.

The possibility of identifying an HLA-DR gene from the product of its expression in transformed cells has several implications. A correlation between the numerous serological allopolymorphic specificities and individual genes of the HLA-D region would represent a major clarification in a system where genetic fine mapping is otherwise impossible. The recent discovery that the human D region is more complex than expected^{16,20,23} makes it imperative to establish the coding

potential and specificity of the product of each of these related but different genes.

One of the most promising applications of the DNA-mediated Ia antigen expression will be in correlations between individual class II genes and their precise functions in the immune response. The exact nature of the gene products recognized by T lymphocytes could be studied with transfected cells expressing products of distinct I or D region genes. Functional assays can now be envisaged, based on the DNA-mediated expression of either one or several class II genes and testing for either allorecognition or Ia-restricted recognition of antigen. In this respect, the combination of the Ia antigen co-transformation system described here (perhaps extended to other cell types) with the availability of specific T-cell clones could be very promising. Finally, the possibility of creating HLA-DR-positive mouse cells by gene transfer introduces a new and unique source of human Ia antigens for the generation of allospecific reagents, including monoclonal antibodies.

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Negatively supercoiled simian virus 40 DNA contains Z-DNA segments within transcriptional enhancer sequences

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Three 8-base pair (bp) segments of alternating purine-pyrimidine from the simian virus 40 enhancer region form Z-DNA on negative supercoiling; minichromosome DNase I-hypersensitive sites determined by others bracket these three segments. A survey of transcriptional enhancer sequences reveals a pattern of potential Z-DNA-forming regions which occur in pairs 50-80 bp apart. This may influence local chromatin structure and may be related to transcriptional activation.

Z-DNA is a left-handed form of the double helix which is favoured in sequences containing alternating purine and pyrimidine residues¹. The structural transition of right-handed

B-DNA to left-handed Z-DNA is energetically favoured in a negatively supercoiled circular double helix²⁻⁴. Recently, a method has been developed² which allows detection and

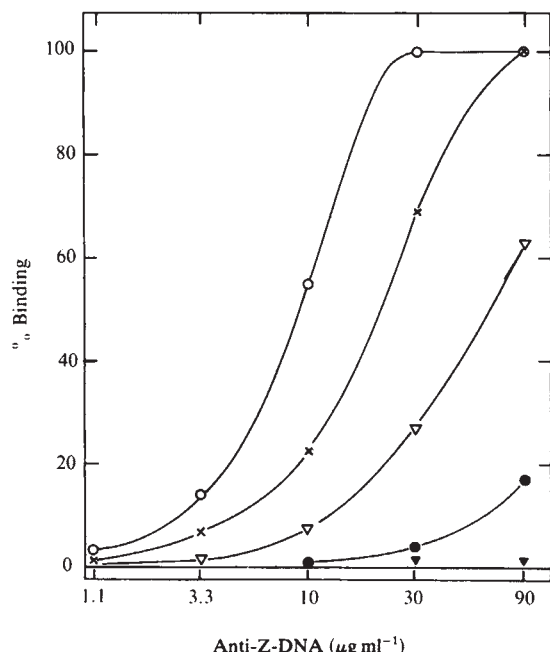


Fig. 1 Anti-Z-DNA antibody binding to supercoiled SV40 DNA. Radioactively labelled ^3H -SV40 DNA was prepared by infection of African green monkey kidney cells (CV1 line) with SV40 strain 777 and viral DNA was prepared as described elsewhere⁵⁰; ^3H -thymidine (10 μl per 25 ml; NEN) was added to the tissue culture medium 24 h before cell collection. Supercoiling of viral DNA and determination of the superhelical densities were carried out as described previously⁴. The specific linking differences (σ) of the DNA preparations were: -0.047 (▼), -0.063 (●), -0.079 (▽), -0.095 (×) and -0.115 (○). Antibody binding was assayed in filter binding tests as described elsewhere², except that PBS buffer (10 mM Na_2PO_4 , 140 mM NaCl, pH 7.4) was used in the binding reaction. Z-DNA formation was assayed using anti-Z-DNA antibodies from purified goat serum, G10c-DEII (purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation and DEAE chromatography). It had anti-Z-DNA reactivity similar to rabbit serum Ra441a (ref. 5). These antibodies are known not to induce Z-DNA formation and perturb the B-Z equilibrium only minimally^{2,5}. The amounts of antibody used are indicated as total purified serum protein per ml reaction volume.

identification of Z-DNA in naturally occurring negatively supercoiled nucleotide sequences by using specific anti-Z-DNA antibodies⁵. Here we show that the negatively supercoiled genome of the papovavirus simian virus 40 (SV40)⁶ forms three major Z-DNA antibody-binding sites within and just outside the 72-base pair (bp) repeat enhancer sequence which is known to have a role in stimulating early viral transcription⁷⁻⁹. A survey of other known enhancer or regulatory sequences reveals DNA segments with the potential to form Z-DNA. These observations may shed some light on the biological activity of transcriptional activator sequences and may have relevance to the general role of Z-DNA in chromatin structure.

Antibody recognition of superhelical SV40

Z-DNA formation in the negatively supercoiled SV40 genome was monitored by binding of antibodies specific for Z-DNA as assayed in filter binding experiments. Radioactive SV40 DNA preparations having varying negative superhelical densities ($-\sigma$) (Fig. 1) were prepared as described in Fig. 1 legend, reacted with anti-Z-DNA antibodies and the retention of DNA-antibody complexes on nitrocellulose filters was measured at increasing concentrations of antibodies. Figure 1 shows that SV40 DNA was not retained when the number of negative superhelical turns was 23.5 or fewer. However, at 31.5 negative superhelical turns, some SV40 DNA was retained on the filter and the retention increased rapidly with increasing negative superhelical density. Over 60% was retained with 39.5

negative superhelical turns and 47 or greater negative superhelical turns produced full retention on the filter. The data plotted in Fig. 1 were obtained at 150 mM Na^+ . Assays carried out at lower cation concentrations yielded a higher level of retention on nitrocellulose filters¹⁰: in 75 mM Na^+ , 50% retention was observed at 23.5 negative superhelical turns. From the data in Fig. 1 we conclude that negative supercoiling of SV40 induces the formation of Z-DNA segments which are recognized by Z-DNA-specific antibodies.

Mapping of antibody-binding sites

To identify the sequences of SV40 which were responsible for forming Z-DNA, the bound antibody molecules were covalently cross-linked to the negatively supercoiled SV40 DNA by glutaraldehyde². The DNA was then digested to completion with excess amounts of multiple-cut restriction endonucleases and passed through a nitrocellulose filter. The DNA fragments cross-linked to antibody were retained on the filter and the filtrate was analysed by polyacrylamide gel electrophoresis to search for partly missing fragments. Initially cross-linked SV40 DNA-antibody complexes were cleaved with *Mbo*II to obtain 16 fragments. Gel analysis of the filtrate revealed a reduced intensity of fragment 3, spanning positions 5,014 to 460 (ref. 11) including the viral origin (data not shown). Double digestion with *Mbo*II and *Pvu*II resulted in reduction of fragment 6 which covers the region 5,014 to 273. A triple digest including the addition of the *Bgl*II restriction endonuclease (single cleavage site at position 0) is shown in Fig. 2, which gives a densitometer scan of the electrophoretically separated filtered cleavage products from protein-free SV40 (top) and SV40-antibody complex (bottom). The more intense peaks are doublets or triplets which were not resolved in the gel. A marked diminution of the 273 bp fragment is apparent. This fragment was derived from the restriction digest between the *Bgl*II site at 0 and the *Pvu*II site at 273 (Fig. 3).

For more precise mapping of the antibody-binding site(s), cross-linked DNA-antibody complexes were subjected to

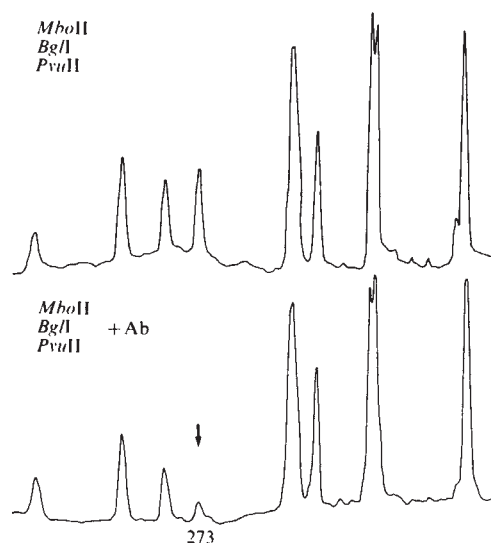


Fig. 2 Identification of the major region in the SV40 genome with potential for Z-DNA formation. Supercoiled SV40 circles ($\sigma = -0.115$) were complexed and cross-linked with anti-Z-DNA antibody as described elsewhere^{2,51}. The cross-linked material was treated with excess amounts of restriction endonucleases *Mbo*II, *Bgl*II and *Pvu*II (all from New England Biolabs) and filtered². The filtrate was concentrated, subjected to 6% polyacrylamide gel electrophoresis and analysed by densitometer scanning as described². Glutaraldehyde cross-linking was performed in the absence (top) and presence (bottom) of anti-Z-DNA-specific antisera (Ab). In the presence of antibody a clear reduction in the scanning intensity can be seen in the 273-bp fragment which is located between the *Bgl*II and *Pvu*II cutting sites. Electrophoretic migration is from right to left.

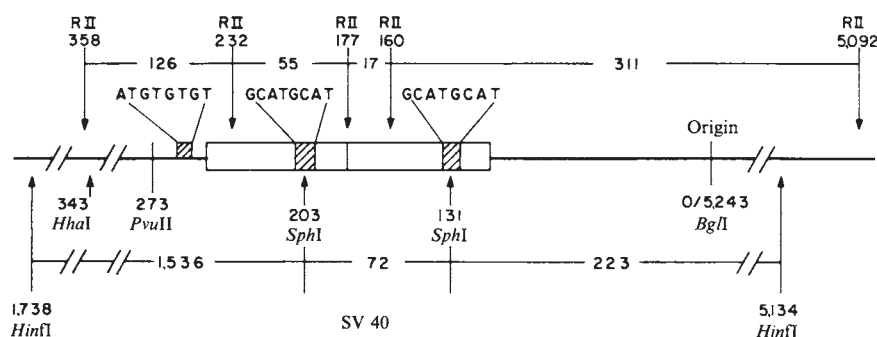


Fig. 3 Location of potential Z-DNA sites in the SV40 origin region. The cross-hatched segments indicate the three major regions that can form Z-DNA in the SV40 genome at map locations 258–265, 199–205 and 128–133. The base sequences of these sites are given. Cutting sites for restriction endonucleases *EcoRII* (RII), *PvuII*, *BglI*, *HhaI* and *HinfI* within this region are indicated, as well as the lengths of some restriction fragments. All map positions are according to the numbering system of ref. 11. The positions of the 72-bp repeat segments are shown as rectangular boxes on the SV40 genome.

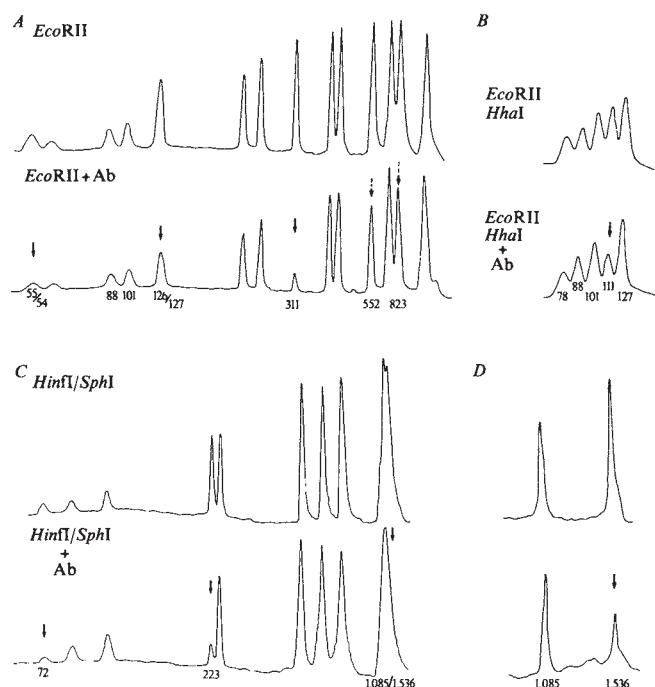


Fig. 4 Mapping of three major sites for Z-DNA formation in the SV40 origin fragment. Supercoiled SV40 DNA was cross-linked without (upper parts of A–D) or with (lower parts) anti-Z-DNA antibodies and analysed as described in Fig. 3 legend after digestion with excess amounts of restriction endonucleases *EcoRII* (A), *EcoRII/HhaI* (B) and *HinfI/SphI* (C, D). Numbers generally refer to the sizes of those fragments that are reduced substantially in scanning intensity; in the *EcoRII* and *EcoRII/HhaI* digests, some additional fragment sizes are shown for orientation. Electrophoresis was carried out in 12% acrylamide (A–C) or 1.8% agarose (D). Solid vertical arrows indicate restriction fragments that are substantially reduced in intensity suggesting the presence of antibody-binding sites; the broken arrows in A indicate fragments that are reduced to a lesser extent.

EcoRII cleavage before filtration, as five *EcoRII* cleavage sites are present in the region of interest (Fig. 3). Figure 4A shows the densitometer tracing of the filtrate of antibody-free (top) or antibody-complexed (bottom) SV40 DNA after *EcoRII* digestion; there are five peaks of decreased intensity. The largest decrease is in the 311-bp fragment (see Fig. 3) as well as in the 54/55-bp doublet which no longer appears as a doublet but rather has an intensity comparable to that of the small singlet peak immediately to its right. In addition, rather smaller decreases are seen in the doublet involving 126/127-bp fragments. Much smaller decreases are also seen for two larger fragments of 475 and 726 bp (dashed arrows). To determine which fragment of the 126/127-bp doublet was reduced due to antibody binding, an additional *EcoRII/HhaI* double digest was performed on antibody-free and complexed SV40 DNA. Figure 4B shows that the 126-bp fragment, which is shortened to 111 bp by *HhaI* cleavage, is reduced in intensity by ~50%.

This identifies an antibody-binding site within the 126-bp *EcoRII* fragment.

Z-DNA is favoured by nucleotide sequences which contain alternations in purine and pyrimidine residues¹. A computer search of SV40 DNA^{12,13} for segments having alternating purine and pyrimidine residues reveals an under-representation of these sequences relative to a random distribution. There are one segment with nine (at 4,580), three segments with eight, and 16 segments with seven alternating purine–pyrimidine nucleotides, respectively. As indicated in Fig. 3, all three of the segments containing eight alternating purine–pyrimidine residues are found near the viral origin, two of them in the repeated 72-bp enhancer segment (indicated by the cross-hatched segments in the rectangular boxes), as well as one in the cross-hatched segment to the left of the 72-bp repeats. We thought these might be candidates for forming Z-DNA which could be responsible for the sharp decrease in the 311- and 55-bp peaks in Fig. 4A as well as the decrease of the 126-bp peak in Fig. 4B.

Experiments were then carried out to determine whether Z-DNA formation could be visualized directly within the 8-bp segment of alternating purine–pyrimidines in the 72-bp repeat sequence. These segments contain the recognition sequence for the *SphI* restriction endonuclease (GCATGC) which does not occur anywhere else in the SV40 genome. Therefore, we analysed the effect of antibody binding to Z-DNA on the cleavage of the *SphI* enzyme as tested in a *HinfI-SphI* double digest. Figure 3 shows that this double digestion should produce fragments of length 1,536, 72 and 223 bp in addition to the other *HinfI* fragments. The results are shown in Fig. 4C; the bottom scan shows the effect of cross-linked antibody on cleavage by the restriction endonucleases. There is a significant reduction in the fragments of 223 and 72 bp, and a reduction in the peak 1,536. The latter shows up less clearly as the initial peak is a doublet containing fragments of 1,085 and 1,536 bp; agarose gel analysis (Fig. 4D) separated these two peaks. A reduction of ~50% in the magnitude of peak 1,536 relative to the 1,085 peak is revealed. These experiments indicate that *SphI* cleavage is blocked to a significant extent by the antibody cross-linked to negatively supercoiled SV40 DNA. From this we infer that, to prevent cleavage by the restriction endonuclease, the antibody binding must be either at or close to the *SphI* recognition sequence. Even though the reduction in the peak intensities in the lower part of Fig. 4 is not uniform, significant inhibition of cleavage by *SphI* is apparent as judged by the decreased intensity of all three peaks. From these studies we conclude that there are three major sites in the SV40 genome with the potential to form left-handed Z-DNA. Two are at the *SphI* cleavage sites within the 72-bp repeat sequence, and a third is probably centred around position 261. Figure 3 displays the identified DNA sequences.

Z-DNA and chromatin structure of SV40

The SV40 minichromosome contains 24 nucleosomes that are unevenly distributed over the genome, leaving a 400-bp nucleosome-free region between the origin of replication and the late region^{14–18}. This area was shown to be nucleosome-free by nuclease digestion^{14–17} and electron microscopy¹⁸ in a significant

Table 1 Potential Z-DNA-forming sequences in regulatory regions

Genome	Sequence	Ref.
SV40 (263)	ATGTGTGT—52—GCATGCAT	11
SV40 (203)	GCATGCAT—65—GCATGCAT	11
Human BK virus	ACATGTCTGT—68—CATGCAC	25
Polyoma (4,447)*	ATGTATGTGT—68—CACACATAT	37
Adenovirus-2, E1A (125)	TGCAAGTGTG—45—GTGTGCGC	38
Adenovirus-2, E1A (125)	TGCAAGTGTG—55—GTGTATACG	38
Bovine papilloma virus (4,400)	GTACACAC—75—GTATAGGTGCG	39
Mouse mammary tumour virus, LTR (4)	CGCGCCTGCA—83—TGCGTGACGCG	40
Mouse mammary tumour virus, LTR (1,138)	TATGTAATATGC—70—CGTGTGTTGTGT	40
Moloney murine sarcoma virus, LTR†	ATATCTGTG—64—ATATCTGTG	41
Murine sarcoma virus, LTR‡	TGTTTCGCGCGC—78—GTACCCGTAT	42
Moloney murine leukaemia virus, LTR‡	GTTCGCGCGC—78—GTACCCGTGTAT	42
Feline leukaemia virus, LTR‡	TGTGCGCGCGC—71—GTACCCGTGTACG	43
AK virus, LTR‡	TGTACCCGCGC—78—GTACCCGTGTAT	42
Simian sarcoma virus, LTR (911)‡	TGTACCCGCGC—69—GTACCTGTGTGT	44
Rous sarcoma virus (SR-A), LTR	CGTGCATGC—60—ACATGGAT	26
Avian myeloblastosis virus, LTR (1,494)	ACATGTATAT—77—GCATATGGGCGT	45
Rous-associated virus-0, LTR (137)	TATGTACG—56—GTGTGCAC	46
Chicken endogenous virus, LTR (114)	ACATATGGGCGTA—73—GTGTGCAC	47
Mouse c-mos oncogene insert (425)	GCACATGCGCA—55—ATGTGGGCGCG	27
Mouse intracisternal A particle, LTR (11)	CGCGCCACAT—55—CGCGCATATGC	28
Mouse intracisternal A particle, LTR (11)	CGCGCCACAT—85—CATGTGCTCATGC	28

The underlined bases are not in an alternating purine–pyrimidine sequence. The number in parentheses designates the position of the first base in the sequence shown, as assigned in the corresponding reference.

* The best candidate for a pair of Z-forming elements in the 244-bp polyoma *BclI/PvuII* fragment which has been implicated in enhancer activity^{48,49}, is the sequence CGTGCG—43—GCGTGTG in strain A2.

† This sequence is conserved in the genomes of AK virus and Moloney murine leukaemia virus.

‡ This pair of potential Z-DNA sites straddles the Goldberg–Hogness (TATA) box and the cap site.

proportion of isolated minichromosomes. The three major sites on SV40 that have potential to form Z-DNA are located within this nucleosome-free region. Recently, proteins have been identified in *Drosophila* nuclei which are able to bind selectively to Z-DNA but not to B-DNA¹⁹. More recent preliminary experiments in our laboratory (F. Azorin, unpublished) indicate the presence of Z-DNA binding proteins in the SV40 minichromosomes. Attachment of such Z-DNA binding proteins to the potential Z-DNA regions might be partly responsible for generating and maintaining the SV40 nucleosome-free region.

Transcriptionally active chromatin contains specific sites having hypersensitivity to DNase I nuclease²⁰. There have been extensive studies of DNase hypersensitivity in the SV40 minichromosome^{14–17,21,22} and in Fig. 5 we have superimposed the hypersensitive sites identified by Yaniv and his colleagues^{21,22} near the 72-bp repeat area onto the Z-DNA-potential regions. The DNase I-hypersensitive sites seem to lie at 25 bp on either side of each possible Z-DNA location. Z-DNA segments that may be associated with specific Z-DNA binding proteins¹⁹, perhaps with locally altered superhelical densities, may represent one type of nuclease-sensitive site.

SV40 enhancer (activator) sequences

The duplicated 72-bp segment near the SV40 origin between bases 106 and 250 has been identified as an essential *cis*-acting site that stimulates early viral transcription^{7–9}. The potential for Z-DNA formation at the transcriptional activator site suggests that Z-DNA formation may be functionally connected with the enhancement activity of the 72-bp repeated sequence. This notion also implies an additional involvement of the 258–264-bp region (the third Z-DNA site outside the 72-bp repeat) in transcriptional activation. SV40 mutants having rearrangements around the 72-bp repeat segment have been investigated for altered enhancer activity^{7,9,23,24}. Analysis of the data shows that SV40 maintains good viability when two of the three major Z-DNA-forming sites are retained in the unaltered form; it also has some limited viability if only one site is maintained. Mutants which eliminate the two Z-DNA regions in the 72-bp repeat retain viability providing the Z-DNA-forming region near 261 remains intact; removal of that third region results in

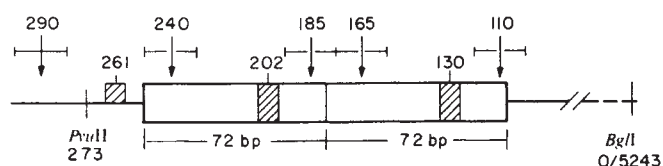


Fig. 5 Correlation between locations of potential Z-DNA sites and DNase I-hypersensitive cutting sites at the SV40 72-bp repeat sequences. Cross-hatched boxes represent the three potential Z-DNA sites centred around positions 261, 202 and 130. The hypersensitive sites for DNase I cutting (ref. 22 and M. Yaniv and S. Cereghini, personal communication) are shown by vertical arrows, with the suggested 10-bp margin of error²² indicated by horizontal bars.

the loss of viability⁹. Similarly, removal of the region near 261 and all of one 72-bp repeat plus part of the next one does not result in total loss of viability unless the Z-DNA-forming region near 132 is also eliminated⁹. These results suggest that one Z-DNA forming site is necessary for virus viability, although three are actually found in the intact SV40 virus. The mutant viruses generally have greater viability if two of the three sites are left intact, in which case the spacing between two such sites seems to be important²³. However, in experiments in which the SV40 enhancer is attached to a globin gene, elimination of two Z-DNA-forming regions results in loss of enhancer activity, although loss of only one of the three regions retains the activity²⁴.

Potential Z-DNA elements in other regions

To obtain information about a possible correlation of Z-DNA-forming ability with enhancer sequences, we examined activator or regulatory sequences of other viral systems for the presence of potential Z-DNA segments having alternating purine and pyrimidine residues. Table 1 lists segments from enhancer sequences and transcriptional control segments of DNA viruses and RNA retroviruses. Significant segments of alternating purine–pyrimidine residues are found which range in length from 8 to 13 bp if there is no more than one bp out of the purine–pyrimidine alternation. Our previous work with negatively supercoiled pBR322 demonstrated that a 14-bp segment of alternating purine–pyrimidine residues with one base pair

out of alternation forms Z-DNA^{2,10}. In view of those results and the results with SV40 presented here it is reasonable to believe that the tabulated sequences of alternating purine-pyrimidine residues have potential for Z-DNA formation. It is remarkable that the potential Z-DNA sites of Table 1 are found as pairs with nucleotide sequences between them varying in length from ~50 to 80 bp. This apparent doubling could be of functional importance and might provide a rationale for the evolution of transcriptional activators as duplicated segments, as is the case for SV40, BK virus and murine sarcoma virus (MSV)²⁵. In the long terminal repeat (LTR) of Rous sarcoma virus (RSV), an enhancer activity has been identified recently²⁶ which has two potential Z-DNA segments 60 bp apart as indicated in Table 1. A 25-bp deletion including the 9-bp Z segment abolishes enhancer activity²⁶. An inserted element was found associated with a recombined cellular oncogene (*c-mos*) in a mouse myeloma tumour²⁷; this has two potential Z-DNA-forming segments 55 bp apart. The 156-bp segment containing these elements has a 90% homology with the LTR of the mouse intracisternal A particle²⁸. It will be of interest to determine whether in these and other viral systems such sequences form Z-DNA segments most readily on negative supercoiling, as is the case for SV40 DNA.

Discussion

The extent of negative supercoiling of SV40 *in vivo* is unclear, although it has been reported that the transcriptionally active fraction of minichromosomes is under torsional strain²⁹. Z-DNA formation *in vivo* is probably stabilized by several factors, including negative supercoiling as well as Z-DNA binding proteins. Segments with alternations of purines and pyrimidines are under-represented in the SV40 genome compared to the expected frequency in a random sequence. Thus it is remarkable that there is a clustering of these sequences in the viral control region containing the only three segments 8 bp long. These are also the three major sites at which Z-DNA forms on negative supercoiling. The presence of similar sequences in other viral systems (Table 1) is striking as all of those examined seem to contain pairs of potential Z-forming segments. The entries in Table 1 generally contain at least 8 bp of alternating purines and pyrimidines with no more than one base pair out of alternation. We have limited our search to 8-bp segments because this is the length which seems to be forming Z-DNA in SV40. It remains to be seen whether segments smaller than 8 bp form Z-DNA.

It is of interest that the region of SV40 which contains the Z-DNA-forming segments is also free of nucleosomes¹⁴⁻¹⁷. Could the attachment of Z-DNA binding proteins be responsible for the absence of nucleosomes in the control region? The correlation with DNase I hypersensitivity shown in Fig. 5 raises the possibility that one class of the hypersensitive sites may be related to the presence of Z-DNA stabilized by Z-DNA binding proteins. However, it is not likely that Z-DNA accounts for all the observed nuclease hypersensitivity in chromatin.

Enhancers have been shown to play a significant part in stimulating transcriptional activity^{7-9,24,30}. However, there has been no obvious common theme associated with these sequences in different viruses to provide a clue as to their function. Here we have observed that the Z-DNA-forming potential of SV40 DNA is greatest in its enhancer sequences. Examination of enhancer sequences in a variety of DNA viruses and retroviral LTRs reveals the presence of similar sequences for which it is reasonable to assume an ability to form Z-DNA. This suggests that the formation of Z-DNA probably with the attachment of Z-DNA binding proteins may have a significant role in the enhancer or activator function. However, it is likely that transcriptional enhancement is a complex process in which several other factors also have a role. Enhancer sequences of some viruses are known to activate transcription with some host specificity³¹. The involvement of Z-DNA formation in transcriptional activation may account for this effect if mediated by sequence-specific Z-DNA binding proteins³².

It is premature to speculate about a detailed mechanism whereby the presence of Z-DNA and Z-DNA binding proteins may be involved in transcriptional enhancement. However, the removal of a Z-DNA protein and the subsequent conversion of left-handed Z-DNA to right-handed B-DNA would be associated with a local increase in negative superhelicity. Changes in superhelicity may represent a form of transcriptional regulation^{33,34}. The presence of pairs of Z-DNA-forming elements raises the possibility that two Z-DNA binding proteins are subunits of a dimer and that a segment of DNA wraps around them with the two Z-DNA-forming elements attached to the protein subunits. If this were the case, such a particle could affect local chromatin structure and generate a recognition signal for an RNA polymerase entry site onto DNA. Once attached to DNA, the polymerase may diffuse to promoter sites³⁵.

The relationship of Z-DNA-forming segments and their possible involvement in transcriptional enhancement can be considered in relation to cellular oncogene activation by RNA retroviruses. Integration of enhancer-associated viral Z-DNA segments may occur independently of orientation both upstream and downstream of cellular oncogenes³⁶. An additional factor in oncogene activation may be the actual length or strength of the Z-DNA potential of a particular regulatory sequence. It is possible that mutations could change the Z-forming potential of the region and consequently modify its behaviour in the control of transcription. The observation of a potential for Z-DNA formation in the SV40 control region should stimulate new experiments to test the possible role of Z-DNA in regulating transcriptional activities.

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LETTERS TO NATURE

Inflation can solve the rotation problem

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If the Universe can rotate, why does it rotate so slowly? We formulate this 'rotation problem' by introducing a dimensionless measure of the angular velocity in the early Universe which must have been $< 5 \times 10^{-28}$ in order to be compatible with present-day upper limits on the rotation. Hitherto the slow rate of rotation has been explained only by invoking Mach's principle, which decrees that it vanish. Here we point out that an inflationary epoch in the very early Universe, proposed as a solution to other cosmological problems, could also solve the 'rotation problem', and thus make the Mach principle redundant.

The list of cosmological problems to be investigated has often included the homogeneity, isotropy, flatness and age of the present-day Universe¹. Why is the Universe now so homogeneous and isotropic on scales which have only recently entered our horizon and were previously held incommunicado by conventional big-bang cosmology? Why is our Universe nearly flat, having an energy density within 1 or 2 orders of magnitude of the critical closure density, despite having lived to the old age of $0(10^{61})$ Planck times? Why is the present-day vacuum energy density so small?

In connection with the observed isotropy, one can be more specific and ask why are the shear² and rotational^{3,4} anisotropies so small today. In general one can add terms to the Einstein field equations which take into account both shear and rotation. The Hubble expansion parameter of an isotropic Universe can be expressed as⁵

$$H^2 = \left(\frac{\dot{R}}{R}\right)^2 = \frac{8\pi G}{3} \rho - \frac{k}{R^2} + \frac{\Lambda}{3} + \dots \quad (1)$$

where R is the Robertson-Walker scale factor, ρ is the mass-energy density, k is the curvature constant ($k=0, \pm 1$ corresponding to a flat, closed or open Universe) and Λ is the cosmological constant. We can add to equation (1) possible terms involving ω (σ), the magnitudes of the rotation (shear) tensors. All of the cosmological problems referred to above can be translated into the single question: why (when they are put into dimensionless forms) are the quantities k , Λ , ω and σ so small today?

We can infer from observation⁶ that the present expansion of the Universe is governed by the matter density (that is we are not now curvature dominated or in a de Sitter space). If we take as a conservative upper limit on the mass energy density $\Omega = \rho/\rho_c < 2$, where $\rho_c \approx 8.1 \times 10^{-47} \text{ GeV}^4 h_0^2$ and $h_0 = (H_0/100) < 1$ where H_0 is the present value of the Hubble parameter in $\text{km s}^{-1} \text{ Mpc}^{-1}$, then the matter density term in equation (1) is bounded by

$$\frac{8\pi}{3} G \rho < 9 \times 10^{-84} \text{ GeV}^2 \quad (2)$$

We must then require that all other terms in equation (1) are also less than the bound in equation (2). For example, the

bound on the cosmological constant (in dimensionless units) becomes

$$\Lambda \leq 2 \times 10^{-121} \quad (3)$$

If Λ is the result of a series of cancellations due to the microphysics at very early times, it represents perhaps the highest degree of fine tuning found in physics today. Of course, there might be some reason why $\Lambda=0$ exactly. But as there is still no satisfactory explanation to this problem, we will not address it any further.

Let us next consider the curvature term in equation (1); this brings about the frequently discussed flatness and age problems¹. Very simply, because it falls off as $1/R^2$ while in the early radiation-dominated era $\rho \sim 1/R^4$, in natural initial conditions $k/R^2 = 0(m_{\text{Planck}}^2)$ at the Planck time, the Universe would have become curvature dominated very quickly. For $k=+1$ this would mean that the Universe would have collapsed long before it had a chance to make a baryon asymmetry. Once again this term must be smaller than the matter density. Since $R \sim T^{-1}$, we can write the dimensionless quantity

$$\hat{k} \equiv \frac{k}{R^2 T^2} \leq \frac{9 \times 10^{-84}}{T_0^2} \leq 2 \times 10^{-58} \quad (4)$$

where $T_0 = 2.7 \text{ K} = 2.3 \times 10^{-13} \text{ GeV}$. This number $\hat{k}$ remains constant during the adiabatic expansion of a Friedmann universe, and hence must have been that small initially. But one might expect that all dimensionless quantities at the Planck time typically took a value $0(1)$.

Possible extra terms in equation (1) describing the rotation (vorticity) and shear are normally neglected. The shear term, however, falls off quite rapidly³ $\sigma^2 \sim 1/R^6$ and put in the form of a dimensionless constant $\hat{\sigma}$ the bound given by equation (2) becomes

$$\hat{\sigma} \equiv \frac{\sigma}{T^3} \leq 6 \times 10^{34} \quad (5)$$

which comfortably accommodates the 'natural' initial value of $0(1)$. A potentially stronger constraint² on σ comes about when one considers the effect of shear on the expansion rate at the time of big-bang nucleosynthesis⁷. In much the same way that the number of neutrino flavours are limited (so as not to overproduce ^4He) the magnitude of the shear tensor can also be limited. One finds² that this corresponds to a present limit

$$\frac{\sigma^2}{3} < 8 \times 10^{-106} \text{ GeV}^2 \quad (6)$$

to be compared with the $9 \times 10^{-84} \text{ GeV}^2$ in equation (2). In constant dimensionless units, however, this only yields the limit

$$\hat{\sigma} \leq 6 \times 10^{23} \quad (7)$$

Analogous arguments applied to the conjectured grand unified theory (GUT) epoch of big-bang baryosynthesis require

$$\hat{\sigma} \leq 0(10^4) \quad (8)$$

Hence because $\sigma \propto 1/R^3$ a value of unity for $\hat{\sigma}$ at the Planck epoch would have reduced to such an extent that shear anisotropy would not interfere at all with big-bang baryosynthesis and nucleosynthesis or our present matter dominated era. Hence there is no 'shear problem'.

We now consider a possible rotation term in equation (2). Its behaviour as a function of T (or R) is slightly more

complicated than that of shear because the rotation also depends on the equation of state⁵. By conservation of angular momentum we have $\rho R^5 \omega = \text{constant}$, or $\omega \sim R^{3\gamma-5}$ where γ determines the equation of state, $p = (\gamma - 1)\rho$. For a matter dominated Universe ($p = 0$, $\gamma = 1$, isotropic pressure) $\omega \sim 1/R^2$ while for a radiation dominated Universe ($p = \rho/3$, $\gamma = 4/3$) we have $\omega = 1/R$. There are therefore two dimensionless constants we must calculate. The first is $m_p \omega / T^2$ which is constant from the time when the energy density of nonrelativistic matter began to dominate over the radiation (approximately the time t_d of photon decoupling) up to today. The second is ω / T which is dimensionless and constant from the Planck era to decoupling in a radiation dominated Friedmann universe. The bound in equation (2) for rotation becomes

$$\frac{\omega}{T^2} \leq 850 \quad (t_d \leq t \leq t_0) \quad (9a)$$

$$\hat{\omega} \equiv \frac{\omega}{T} \leq 6 \times 10^{-25} \quad (t_p \leq t \leq t_d) \quad (9b)$$

where $t_p \approx \frac{1}{2} \times 10^{-43}$ s is the Planck time, $t_d \approx 10^{12}$ s is the time of decoupling and $t_0 \approx 3 \times 10^{17}$ s is the present age of the Universe.

If one considers^{3,8} the effect of rotation on the microwave background radiation, one obtains the limit $\omega \leq 1 \times 10^{-21} \text{ s}^{-1}$. For larger vorticities, we would have an unacceptably large apparent peculiar velocity. Using this limit one obtains

$$\frac{\omega}{T^2} \leq 0.15 \quad (t_d \leq t \leq t_0) \quad (10a)$$

$$\hat{\omega} \leq 10^{-28} \quad (t_p \leq t \leq t_d) \quad (10b)$$

A significantly stronger constraint on the amount of rotation at present comes from looking at the effect of the shear induced by the rotation⁹. For rotation on a scale λ , the amount of shear induced is given roughly by $\sigma \sim \lambda^2 T \dot{\omega} / 2t^2 \approx 6 \times 10^{-36} \lambda^2 T^5 \dot{\omega}$ (using the time-temperature relation $T^2 \approx 3 \times 10^{17} \text{ GeV}$ in a radiation dominated epoch). If we consider rotation on the scale of the horizon today then $\lambda T \approx 1.5 \times 10^{29}$. The induced shear is then $\sigma \approx 10^{23} T^3 \dot{\omega}$. In order that the shear does not dominate at the time of baryosynthesis, we must require that

$$\sigma^2 \approx 10^{46} T^6 \dot{\omega}^2 < \frac{8\pi}{m_p} \rho(T_{\text{GUT}}) \quad (11)$$

which for $T_{\text{GUT}} \approx 10^{15} \text{ GeV}$ implies that

$$\dot{\omega} \leq 3 \times 10^{-56} \quad (12)$$

Even if we relax this constraint so that the shear does not dominate at the time of nucleosynthesis

$$\dot{\omega} \leq 3 \times 10^{-39} \quad (13)$$

These limits correspond to $\omega < 3 \times 10^{-49} \text{ s}^{-1}$ and $\omega < 3 \times 10^{-32} \text{ s}^{-1}$ respectively, which means that rotation on or near the horizon scale should not now be observable. The question we pose here as the 'rotation problem' is: why was the value of $\hat{\omega} \equiv \omega / T$ as small as $0(10^{-56})$? Again we might naively expect a value of the order of 1 for $\hat{\omega}$ at the Planck epoch.

Before one panics at the sight of yet another cosmological problem, it is worth asking whether or not the effects of viscosity could damp an initially large vorticity. In the presence of a shear viscosity, the vorticity will be damped³ by

$$\exp\left[-16\pi \int \eta dt\right] \quad (14)$$

where η is the coefficient of shear viscosity¹⁰

$$\eta = \frac{4\pi^2}{450} N(T) T^4 \tau \quad (15)$$

Here $N(T)$ is the number of interacting degrees of freedom and τ is the typical mean free time. There were perhaps three

different epochs during which one might have expected a damping possibility. The last was during the period of decoupling. In this case, $N(T) = 2$, and $\tau = (n_\gamma \sigma_T)^{-1}$ where $n_\gamma \approx 0.24 T^3$ and the Thompson cross-section is just $\sigma_T \approx 1.7 \times 10^3 \text{ GeV}^{-2}$ so that $\eta = 4.27 \times 10^{-4} T$. Conservatively, we might say that the viscosity occurred at $T = 10^5 \text{ K}$ for a period of time lasting 10^5 yr . In this case, the damping factor (14) is only $\exp[-2 \times 10^{-12}]$ which is negligible. One might also expect viscosity due to neutrinos¹¹ to be important in the lepton era. We use the weak interaction cross-section $\sigma_{\text{weak}} = G_F^2 T^2$ so that $\eta \approx 10^{10} / T$. Using the relation $t \approx 10^{18} / T^2 \text{ GeV}^{-1}$ and integrating up to the time of neutrino decoupling, we find that the factor (14) is at most $0(1)$ [in agreement with Hawking's analysis³]. Finally, one might expect a contribution to the viscosity damping¹² some time during the grand unified era. In this case it has been estimated that $\eta = 650 T^3$ and integrating over a period¹³ $\Delta T \approx 10^{15} \text{ GeV}$ we find once again that the damping factor (14) is only about $0(1/10)$. In any case, we do not expect viscous damping effects to be significant on scales larger than the horizon size. Hence we conclude that an initial vorticity would not be damped by shear viscosity and we are left with the rotation problem.

One could choose to ignore this problem, as was done until recently for many other cosmological problems. Or one might simply invoke Mach's principle¹⁴ which would guarantee that $\hat{\omega} = 0$. However, many cosmological problems can be resolved by microphysics^{1,15}, especially if there is a supercooled phase transition during which the Universe passed through a de Sitter or exponentially expanding period at an early stage of its evolution. During such a stage, the dimensionless quantities introduced in equations (4)–(10) are no longer constant. Instead, they fall off exponentially in ways related to their dependences on R , and can be reduced from unity to observationally acceptable values.

Let us now consider the effect of inflation on rotation. At first sight one might conclude that unless $\hat{\omega} < 0(10^{-56})$ before the onset of inflation, the Universe would have been shear dominated and might never have passed through the inflationary phase¹⁴. This problem, of course, stems from the fact that we have chosen the scale λ to match the horizon today. In a conventional Friedmann universe (a perturbation now on this horizon scale would have been many orders of magnitude larger than the horizon at the Planck epoch. Suppose that instead we choose the 'natural' initial conditions $\hat{\omega} = 1$, $\lambda T \approx 1$, then the shear induced will be $\hat{\sigma} \approx 10^3$ and cannot dominate at any time at or after the GUT epoch. If there was inflation, this small scale perturbation could get blown up to be larger than the horizon of the Universe. During this inflation, $\omega \sim 1/R$, so that if the Universe inflated by $0(10^{30})$ then the scale λ would become roughly the present horizon scale but $\hat{\omega}$ will have reduced to $0(10^{-30})$. Today this corresponds to the possibly observable value of $\omega \leq 10^{-23} \text{ s}^{-1}$.

However, for this inflationary scenario to work, it is necessary that the Universe did not become curvature, or rotation dominated before the inflationary epoch got underway. Therefore one needs initial values

$$\hat{k}_i < 0\left(\frac{T_{\text{DS}}}{T_p}\right)^2, \quad \hat{\omega}_i < 0\left(\frac{T_{\text{DS}}}{T_p}\right) \quad (16)$$

where T_{DS} is the temperature at which the vacuum energy driving the de Sitter expansion starts to dominate the mass-energy density ρ in the expansion equation (1). If $T_{\text{DS}} = 0(10^{-3}-10^{-4}) T_p$ as in many inflationary scenarios based on GUT, then equation (16) tells us that we still need some finetuning of $\hat{k}$ and $\hat{\omega}$ to small values¹⁶, the reason being, that for values of $\hat{k}$ and $\hat{\omega}$ larger than in equation (16), the Universe might not have had the chance to inflate. This is especially true for $k = +1$ where the Universe would recollapse before inflation. Because of the sign of the possible ω term in equation (1), rotation could also produce the same effect. However, these modest versions of the problems can also be avoided if inflation took place at the Planck epoch¹⁷: $T_{\text{DS}} \approx T_p$. We do not need to

worry about shear dominating the energy and preventing inflation in an analogous way. Because the shear-energy density falls rapidly with T , it will eventually become negligible to the vacuum-energy density, and inflation will ensue¹⁸, even if its onset is belated.

The cosmological inflation introduced¹ for other reasons may, therefore, also solve the rotation problem. [This solution is part of Guth's original motivation for inflation, it can also be regarded as a consequence of the "cosmic no-hair" theorem of Hawking and Moss¹⁹.] In this sense it makes Mach redundant, because we no longer need to invoke his principle¹⁴ to understand why distant objects rotate so slowly relative to a bucket of water whose surface is flat, or a ballet-dancer whose hands fall limply at his sides¹⁰. Moreover, inflation offers the possibility that Mach's principle might be slightly false. Unfortunately, it suggests that distant objects are likely to rotate at an unobservably slow rate relative to a truly flat water-bucket or a truly limp ballet-dancer.

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111-day periodicity of X-ray transient A0535+26

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A0535+26 is the prototype object of the hard spectrum, pulsating X-ray transients associated with Be companions. Our analysis of its 1969–79 X-ray (3–12 keV) time history, measured by the Vela 5B satellite, shows a period of 111.0 ± 0.4 days, confirming the suggestion of Nagase *et al.*^{1. A likely explanation for the period is binary motion in a wide orbit. 'Giant' outbursts (flux ≥ 1 Crab ≈ 40 Vela counts s^{-1}) observed in 1975 and 1980 are not in phase with the 111-day maxima. The source became significantly more active after the 1975 event, averaging ≈ 3 Vela counts s^{-1} , compared with ≈ 0.5 counts s^{-1} before. The latter count rate corresponds to a time averaged X-ray luminosity of 1×10^{35} erg s^{-1} at 1.3 kpc. The mass loss rate from the companion, HDE245770, required to power this luminosity by wind accretion is $10^{-5} M_{\odot} yr^{-1}$, which is excessive for a B0V star. It is more likely that the transferred matter is lost from the equatorial region of the rapidly rotating B star.}

Binary systems consisting of neutron stars and emission B star (Be) companions, in wide orbits, are a major class of bright galactic X-ray sources. These objects are characterized by hard

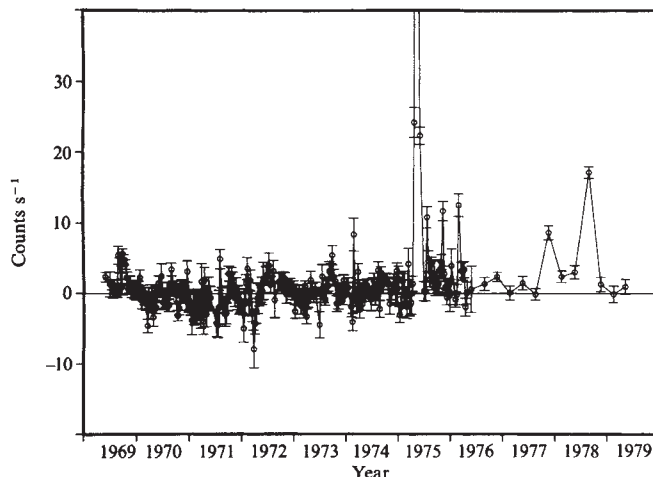


Fig. 1 The 3–12 keV time history of A0535+26 for the period 1969–79. Each point represents 10 days of data (90 days after mid-1976). The giant outburst of April 1975 is off scale, peaking at 113 counts s^{-1} . A few poorly determined points (1σ error > 3.5 counts s^{-1}) are not plotted for clarity.

spectra, irregular or transient activity, and are often X-ray pulsars (for a review see ref. 2). The binary periods of Be/X-ray systems are a few weeks or longer, and are difficult to determine. Eclipses are unlikely, and Doppler pulse timing analysis is complicated by intrinsic variations in the pulse period. A0535+26 was discovered during an outburst in April 1975, during which it attained an intensity more than double that of the Crab nebula³. X-ray position data led to the identification of its companion HDE245770, a rapidly rotating B0Ve star⁴. Analysis of pulse timing data ($P = 104$ s) for the effects of binary motion have not yielded a binary period; the lack of Doppler variations excludes periods < 20 days (ref. 5). Nagase *et al.*¹ report that the nine outbursts observed since April 1975 suggest a 111-day period. If real, the outburst period is probably the binary period of the system, as has been demonstrated for the Be/X-ray system GX301–2 (refs 6, 7). The X-ray outbursts may be modulated by the varying separation in an eccentric orbit.

A small scintillator–photomultiplier scanning detector on the Vela 5B spacecraft provided 3–12 keV all-sky coverage for the period 1969–79, with incomplete data acquisition after June 1976. The $6.1^\circ \times 6.1^\circ$ (FWHM) collimator pointed perpendicular to the spin axis of the Earth-oriented spacecraft, which had a nominal spin period of 64 s. The celestial sphere was thus scanned twice per 112-h orbit. Sensitivity is limited by the detector background, which was ~ 0.8 Crab. The 27 cm^2 detector yielded ≈ 40 counts s^{-1} for the Crab, so that 1 Vela count $s^{-1} \approx 25$ UFU $\approx 6 \times 10^{-10}$ erg $cm^{-2} s^{-1}$ (2–11 keV) for a source with the spectrum of the Crab⁸. This corresponds to $\approx 4.5 \times 10^{-10}$ erg $cm^{-2} s^{-1}$ in the 3–12 keV response band of the Vela detector.

Scanning data were summed into all-sky maps. Since aspect information is good to $\pm 0.2^\circ$, the maps can be fit to the collimator response for point sources, to determine the intensity of overlapping sources. To obtain source time histories, we fit the region around the Crab and A0535+26 (4° away) to the intensity of the two sources and background terms. The experiment and analysis technique are described in more detail in refs 9, 10 and refs therein.

Figure 1 shows the 1969–79 3–12 keV time history of A0535+26, with 10-day time resolution (90 days after June 1976). The April 1975 outburst is off-scale, peaking at 113 counts s^{-1} . Also apparent are the previously reported outbursts of July and November 1975, December 1977, and August 1978 (see ref. 1 and refs therein). A local maximum of 3.1 ± 1.5 counts s^{-1} occurs for JD2440940–0950, which is the time of the UHURU pre-discovery detection¹¹ (≈ 13 UFU) of 27 December 1970. Previously unreported outbursts, apparent in September–October 1969 to ≈ 0.1 Crab, July 1972 (≈ 0.1 Crab),

September 1973 (≈ 0.1 Crab), and February 1976 (≈ 0.3 Crab) fall at maxima of the 111-day cycle determined below. The high point of February 1974 is out of phase. Note that no giant outbursts (≥ 1 Crab, as in April 1975 and September 1980¹) occurred in the six pre-discovery years 1969–75.

Fourier analysis of the time history demonstrates the existence of the 111-day period. Figure 2 shows the Fourier transform of the data of Fig. 1, omitting the 90-day points and data from the April 1975 'giant' outburst. Data were weighted by their error bars before transformation. The transform includes a peak at 1 cycle yr^{-1} , which is almost certainly spurious. This effect is likely to be due to a small annual error in aspect determination, because the intensity deconvolution depends on accurate aspect, which was determined by a Sun sensor. Even a slightly incorrect aspect leads to source contamination from the Crab, 4.4° away. The 1 cycle yr^{-1} peak corresponds to an annual variation of $\pm 1 \text{ count s}^{-1}$, which would be caused by an aspect error of $\pm 10 \text{ arc min}$. An error of this magnitude is not unexpected. Because the source lies near the ecliptic, loss of data every June when the Sun is nearby yields an annual contribution to the power spectrum window function.

Annual effects aside, the power spectrum is dominated by power at $\nu = 3.29 \text{ yr}^{-1}$ and its first harmonic (for our purposes, $1 \text{ 'Julian' yr} \equiv 365.25 \text{ days}$). The 1 cycle yr^{-1} contamination is the cause of the side bands at $\nu + 1$, $2\nu - 1$, and $2\nu + 1$; actual noise peaks are smaller. The peak at $17.8 \text{ cycles yr}^{-1}$ is not significant; the probability of such a peak occurring at a random frequency in the spectrum is 50%. The fundamental and first harmonic stand at 9 and 6 times the local mean power, with random probabilities of 10^{-4} and 3×10^{-3} of occurring at a given frequency, and have maxima which agree in epoch. Since we are testing a suggested period, rather than searching a wide range of frequency space, the significance of the periodicity is convincing. Note that the time span tested has very little overlap with the outbursts tabulated by Nagase *et al.*¹.

The best fit period is $111.0 \pm 0.4 \text{ days}$ (1σ), with maxima at $\text{JD}2441728 (\pm 4) + nP$. The folded light curve is inset in Fig. 2; the shape of the curve confirms the reality of the first harmonic in the Fourier transform. Nagase *et al.*¹ estimate that periastron occurs between $\text{JD}2444510$ and 2444525 , assuming a 111-day orbit; photometric maximum thus falls $0.24\text{--}0.02$ cycles before periastron, suggesting that outbursts are related to enhanced mass accretion at closest approach. The giant outbursts in April 1975 and September 1980¹ appear out of phase with the 111-day period, occurring at phases $0.17\text{--}0.59 (\pm 0.04)$ and $0.05\text{--}0.40 (\pm 0.10)$ respectively, suggesting an origin different from the smaller events. The seven smaller outbursts tabulated by Nagase *et al.*¹ have times of maxima consistent with our ephemeris.

The time-averaged luminosity of A0535+26 increased after the April 1975 event. Excluding the outburst itself, the mean flux June 1969–April 1975 is $0.5 \text{ counts s}^{-1}$, while the May 1975–June 1979 flux is $3.0 \text{ counts s}^{-1}$. The difference is probably explained by the greater source activity after 1975 (see Fig. 1). A lower limit to the mean source flux for the period 1969–76, independent of any possible error in background subtraction, can be obtained from the light curve. The 'pulsed flux' present in the folded light curve inset to Fig. 2 (assuming the mean of the six lowest bins is baseline) is $0.50 \pm 0.06 \text{ counts s}^{-1}$, consistent with the weighted mean of $0.6 \text{ counts s}^{-1}$ for the total flux over the same period. An average flux of $0.5 \text{ counts s}^{-1}$ implies a $2\text{--}11 \text{ keV}$ X-ray luminosity of $1 \times 10^{35} \text{ erg s}^{-1}$ for a source distance of 1.3 kpc (ref. 4). Even when the source does not show major outbursts, as in the period June 1969–April 1975, the minor activity which shows a 111-day period produces a substantial time-averaged luminosity.

The May 1975–June 1979 mean luminosity is consistent with the secular spin-up observed by Nagase *et al.*¹. The accretion torque model of Ghosh and Lamb¹² predicts a spin-up rate $\dot{P} = -4 \times 10^{-2} \text{ s yr}^{-1}$ for $L = 6 \times 10^{35} \text{ erg s}^{-1}$. Nagase *et al.*¹ and Li *et al.*⁵ report intervals of both spin up and spin down from May 1975 to September 1980, but the mean spin-up rate

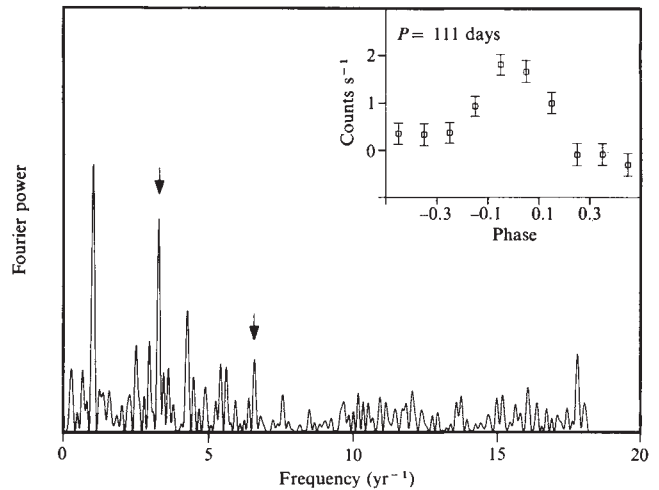


Fig. 2 Fourier transform of the data of Fig. 1. The 90-day points and the April 1975 outburst were edited out before transformation. Arrows indicate $P = 111$ day and its first harmonic. The inset shows the folded light curve for a 111-day period.

$\dot{P} \approx 3 \times 10^{-2} \text{ s yr}^{-1}$ is consistent with the predicted value (see their Fig. 3). The smaller flux for June 1969–April 1975 implies $\dot{P} \approx 1 \times 10^{-2} \text{ s yr}^{-1}$ for that period; higher time resolution analysis of our September–October 1969 data may test this prediction.

White *et al.*¹³ argue that the X-ray luminosities of the faint, nearby Be/X-ray systems X Per and γ Cas are too high to be explained by accretion from the observed stellar winds, and therefore require mass loss from the equatorial region of the Be stars. The discrepancy is even more severe for A0535+26, since it has a much greater X-ray luminosity than X Per and γ Cas, which average 4×10^{33} and $1 \times 10^{33} \text{ erg s}^{-1}$, respectively. We calculate below the wind mass flux required to feed the X-ray source. Assuming spherical symmetry, the wind flux as sampled in the orbital plane is

$$\dot{M} = 2 \times 10^{-9} (1 - \epsilon^2)^{-1/2} L_{35} \left(\frac{R_x}{10 \text{ km}} \right) \times M_{1.4}^{-3} v_3^4 P_d^{2/3} \left(\frac{M_T}{M_\odot} \right)^{2/3} M_\odot \text{ yr}^{-1}$$

where L_{35} is the X-ray luminosity in $10^{35} \text{ erg s}^{-1}$, R_x is the radius of the neutron star, $M_{1.4}$ is its mass in units of $1.4 M_\odot$, v_3 is the wind velocity in units of $1,000 \text{ km s}^{-1}$, M_T is the total system mass, ϵ is the orbital eccentricity, and P_d is the binary period in days. The equation is the same as that of White *et al.*¹³ except for the $(1 - \epsilon^2)^{-1/2}$ term, which corrects for the time average of r^{-2} in an eccentric orbit. For $\epsilon \leq 0.87$, the eccentricity term has less than a factor of 2 effect. For a 110-day orbit, and assuming $L_{35} = 1$, $M_T = 20 M_\odot$, $R_x = 10 \text{ km}$, $\epsilon = 0$, and $M_{1.4} = 1$, $\dot{M}$ is $10^{-5} M_\odot \text{ yr}^{-1}$. In contrast, the expected wind mass loss rate for a B0V or B0Ve star is $\approx 5 \times 10^{-9} M_\odot \text{ yr}^{-1}$ (refs 14, 15). A wind velocity $v_3 = 1$ is at the lower end of the range for a B0V star (for a discussion of wind velocity data, see ref. 13). The wind flux required to power A0535+26 thus exceeds by a factor of $>1,000$ the expected value. Be stars show spectroscopic signs of mass loss from their rapidly rotating equatorial regions¹⁶; the greater rate, and perhaps lower terminal velocity, of loss from these regions may suffice to power A0535+26.

The Vela 5B X-ray experiment was designed and implemented by W. D. Evans, J. P. Conner, R. D. Belian, J. A. Bergey, H. C. Owens, and E. R. Tech of Los Alamos, plus the staff of Sandia National Laboratory. We thank R. Kelley for suggesting that we examine our A0535+26 data for 111-day periodicity. F. Cordova and E. Fenimore made useful comments on the manuscript. This work was performed under the auspices of the US Department of Energy.

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On neutron star structure and the millisecond pulsar

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The recently discovered millisecond pulsar¹ (PSR1937+214) has been observed to be rotating close to the limit of dynamic instability for a neutron star. In spite of its extremely rapid rotation, the present measurements of the period derivative^{2,3} put a stringent upper limit on the energy loss from gravitational radiation, thus requiring that the quadrupole moment be quite small. The pulsar must also be rotating below the critical frequency at which its equilibrium configuration would become non-axisymmetric, since the lifetime of this configuration against decay by gravitational radiation is very short. This critical frequency, given by the theory of rotating ellipsoids⁴, imposes a more severe restriction on the rotation rate than the break-up frequency and may be used to set a lower limit, $\langle \rho \rangle > 2 \times 10^{14} \text{ g cm}^{-3}$, on the density of the star. If the mass is $0.5\text{--}1.5 M_{\odot}$, several of the stiffer neutron star equations of state may be ruled out, and the radius should be $< 16 \text{ km}$. The condition for axisymmetry also imposes an upper limit on the rotation rate to which neutron stars may be spun up by accretion disks in binary systems, a model recently proposed for the evolution of the millisecond pulsar⁵.

Rigidly-rotating masses deform into non-spherical equilibrium shapes, whose geometry becomes increasingly complex at rapid rotation rates. A homogeneous, uniformly-rotating body will deform (in the newtonian approximation) into an oblate spheroid with eccentricity dependent only on rotation rate Ω and density ρ . At high rotation rates, the equilibrium figure can become triaxial at the point where the Jacobi ellipsoids, having lower Ω at a given eccentricity, bifurcate from the axisymmetric Maclaurin sequence. This bifurcation point, for a homogeneous body, is⁴

$$\frac{\Omega_{\text{crit}}^2}{\pi G \rho} = 0.374 \quad (1)$$

Above this point, and below the bifurcation points of more complex ellipsoids, there are actually two possible equilibrium configurations with different Ω at a given eccentricity. It has been shown, however, that the Maclaurin spheroids are secularly unstable from either viscosity or gravitational radiation reaction at the bifurcation point⁶. Therefore, masses rotating with $\Omega > \Omega_{\text{crit}}$ are expected to become Jacobi ellipsoids. But because of its quadrupole moment, a Jacobi ellipsoid will relax by gravitational radiation to a Maclaurin spheroid with $\Omega = \Omega_{\text{crit}}$ by spinning up (but losing angular momentum by decreasing its moment of inertia) on a time scale of $\sim 0.1 \text{ s}$ (ref 7).

There is reason to believe that rapidly rotating neutron stars may deform into triaxial ellipsoids. Their rigidity is high and, with the exception of possible (small) slippage between the

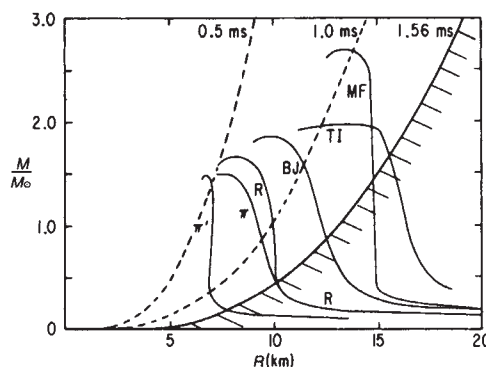


Fig. 1 Gravitational mass versus radius (from ref. 14) for a variety of neutron star equations of state. Sources are, beginning with the softest equations, π' , π (ref. 15), R (ref. 16), BJ (ref. 17), MF (ref. 18), TI (ref. 19). The hatched line denotes the mean density cutoff at $\langle \rho \rangle = 2 \times 10^{14} \text{ g cm}^{-3}$, imposed by the rotation of the millisecond pulsar. The dashed lines are density lower limits which would be set by shorter period pulses.

superfluid core and the crust, the star is thought to be uniformly rotating. Homogeneity is probably a good approximation for neutron stars with masses greater than around $0.5 M_{\odot}$, whose density is fairly uniform from the centre out to the last few metres of crust. Low-mass neutron stars have large radii, thick crusts and density profiles which are better modelled by polytropes of index $n > 1$. Equilibrium figures for polytropic models have been investigated⁸, and it has been shown that Jacobi ellipsoids exist for a polytropic index $n < 0.8$, which would correspond to neutron stars of higher mass. By assigning polytropic indices to neutron star models, Tsuruta and Cameron⁹ conclude that neutron stars with $M \geq 0.5 M_{\odot}$ can deform into triaxial ellipsoids. If, at some stage in its evolution, a pulsar is spinning rapidly enough to become non-axisymmetric, however, it would not have much chance of being observed, because of the short lifetime of the Jacobi ellipsoid phase. Therefore, Ω_{crit} may be considered an upper limit to observable pulsar rotation rates, unless pulsars are very low mass neutron stars.

The millisecond pulsar, with a period of $P = 1.558 \text{ ms}$ ($\Omega = 4,033 \text{ s}^{-1}$), is the first observed example of a neutron star spinning rapidly enough to approach the Jacobi bifurcation point and thus affords the possibility of constraining neutron star physics. From the presently measured value of the period derivative, $\dot{P} = 1.2 \times 10^{-19} \text{ s s}^{-1}$ (refs 2,3), the pulsar is losing energy at a rate $\dot{E} = 1.2 \times 10^{36} \text{ erg s}^{-1}$, only 2×10^{-3} times that of the Crab pulsar. Even if all of this energy loss is gravitational radiation, the star must be close to axisymmetry, with an eccentricity $e = D_{\perp}/17 I_{45} < 4 \times 10^{-9}$, where $D_{\perp}$ is the quadrupole moment perpendicular to Ω and I_{45} is the moment of inertia in units of 10^{45} g cm^2 . Because quadrupole moments can also result from anisotropic pressure and magnetic field stresses¹⁰, this is only an upper limit to rotationally induced axisymmetry. Regardless of the origin of the rapid rotation rate of the pulsar, from equation (1) it must presently have $\Omega < \Omega_{\text{crit}} = (0.374 \pi G \rho)^{1/2}$. Its mean density must therefore satisfy $\langle \rho \rangle > 2 \times 10^{14} \text{ g cm}^{-3}$, in the approximation of homogeneity. Some neutron star equations of state predict mean densities below this value for certain values of the mass. Figure 1 shows models for neutron stars calculated from a variety of equations of state, ranging from very soft to very stiff dependences of pressure on density. The soft equations of state, including the pure neutron (R) and pion condensate cores (π, π'), require higher densities to provide the pressure necessary to hold the star up against collapse. The stiff equations of state, however, allow lower densities which appear to be incompatible with the condition above for masses below $1.5 M_{\odot}$. For neutron stars with $M \leq 0.5 M_{\odot}$, which may not deform into triaxial ellipsoids, the limit on Ω would not apply. If the millisecond pulsar has a mass in the range $0.5\text{--}1.5 M_{\odot}$, then, the stiff equations of state can be

ruled out. It would, of course, be interesting to investigate the equilibrium figures of rotating neutron stars with these equations of state to verify the above result.

Several evolutionary scenarios have been proposed for this pulsar, and these models predict a whole class of short period pulsars with low magnetic fields. Alpar *et al.*⁵ suggest that neutron stars in binary systems may be spun up by keplerian accretion disks. The minimum period is the period of the keplerian orbit at the Alfvén radius, so that

$$P \geq 6 \times 10^{-4} B_8^{6/7} R_6^{18/7} (M/M_\odot)^{-5/7} \dot{M}_{17}^{-3/7} \text{ s} \quad (2)$$

where B_8 is the surface dipole field in units of 10^8 G, R_6 is the stellar radius in units of 10^6 cm, M is the mass and $\dot{M}_{17}$ is the accretion rate in units of 10^{17} g s^{-1} . Neutron stars with weak magnetic fields should therefore have the shortest periods. However, the star can only be spun up to the point of becoming non-axisymmetric, $P_{\text{eq}} = 1 \times 10^{-3} (M/M_\odot)^{-1/2} R_6^{3/2} \text{ a}$ [using equation (1)], beyond which the excess kinetic energy of rotation will be released as gravitational radiation. This will be the case for neutron stars with magnetic fields,

$$B_8 < 1.8 R_6^{-5/4} (M/M_\odot)^{1/4} \dot{M}_{17}^{7/9} \quad (3)$$

As the accretion continues, the star will remain at the equilibrium period P_{eq} , emitting gravitational radiation at a rate equal to the rate of transfer of rotational kinetic energy from the disk,

$$\dot{E}_{\text{grav}} = I \Omega_{\text{eq}} \dot{\Omega} \\ = 4.4 \times 10^{36} I_{45} (M/M_\odot)^{1/2} R_6^{-3/2} B_8^{2/7} \dot{M}_{17}^{6/7} \text{ erg s}^{-1} \quad (4)$$

where I_{45} is the moment of inertia, and the rate of spin-up is that given by Ghosh and Lamb¹¹.

Alternatively, the pulsar could have been born in isolation with a very weak magnetic field, as has been proposed by Brecher and Channugam¹². In this case, if the initial spin were beyond the critical bifurcation frequency, the star would have quickly relaxed back to Ω_{crit} through gravitational radiation.

Discovery of a shorter period pulsar could place further constraints on neutron star structure. For example, the two stiffest equations of state (MF and TI) could be eliminated completely for $M \geq 0.5 M_\odot$ by the existence of a pulsar with a period ≤ 0.9 ms, and the Bethe-Johnson equation (BJ) would be restricted to either very low or very high masses. Since the BJ equation of state is currently believed to be the most realistic, the discovery of a pulsar with $P \leq 1$ ms would have very interesting implications for neutron star structure. The constraint on certain equations of state may have consequences for calculations, such as neutron star cooling times, which have been found to depend on the structure of the star¹³.

I thank D. Kazanas and S. Tsuruta for discussion and comments.

Note added in proof: Since submission of this letter, I have received a preprint from Shapiro *et al.*²⁰ who, from the rotation of the millisecond pulsar, have independently come to similar conclusions about limits on neutron star equations of state.

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Direct optical observation of ferromagnetic domains

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We report here the first direct optical observation in any material of ferromagnetic domains using the magnetostrictive effect. The domains were seen, using an optical microscope at near normal incidence, on the (110) surface of a crystal of terfenol ($\text{Tb}_{0.27}\text{Dy}_{0.73}\text{Fe}_2$), which possesses a very large magnetostriction constant ($\lambda_{111} = 1.6 \times 10^{-3}$ at room temperature). Magnetostrictive distortions of adjacent domains in the specimen caused the surface to take up a rippled appearance. The application of a constant magnetic field of 3×10^{-2} T in the [111] direction resulted in the growth of the domain pattern, and confirmed that the surface undulations were indeed due to ferromagnetic domains.

It is just over 75 yr since Weiss¹ postulated the presence of an internal field to account for the large spontaneous magnetizations found in ferromagnetic materials. At the same time, to explain how a ferromagnetic material could be demagnetized, he also suggested the existence of magnetic domains. Within each domain the material possesses a spontaneous magnetization but because the orientation of the magnetization vector differed in the various domains, the net magnetic moment of the bulk material could be zero. The presence of such magnetic domains was inferred in the classical experiments of Barkhausen², and in the 1930s individual domain boundaries were revealed by use of magnetic colloid deposition techniques³. Since then a variety of methods have been developed for the imaging of domains and domain boundaries including magneto-optical, electron-beam⁴ and X-ray and neutron-topographic techniques⁵.

When a solid orders ferromagnetically the crystal lattice spontaneously distorts in the magnetization direction. This distortion is known as the magnetostriction. It follows that a pair of domains with their magnetization directions inclined to one another will have lattice distortions that are also mutually inclined to one another. To preserve lattice coherency at the boundary, there exists a lattice tilt across the domain wall analogous to that at a coherent twin boundary. It has long been appreciated that for some domain configurations, these lattice distortions will give rise to macroscopic tilts of the specimen surface. Although these tilts should be, in principle, detectable optically they are typically seconds of arc and their optical observation has not been reported previously. The recent availability of good quality single crystals of terfenol ($\text{Tb}_{0.27}\text{Dy}_{0.73}\text{Fe}_2$), has enabled us to see this effect for the first time.

Terfenol has the cubic MgCu_2 type of structure⁶ with (111) easy magnetization directions. The magnetostrictions at room temperature are 10–100 times greater than those of nickel, and hence the lattice tilts are of the order of arc minutes. The specimen on which our observations were made was cut by electro-spark erosion and was disk-shaped with a diameter of ~10 mm and a thickness of ~4 mm. A mirror finish was obtained on one of the faces of the disk by careful mechanical and chemical polishing. The final orientation of this surface was, to within a few degrees, parallel to the (110) plane.

Figure 1 shows optical micrographs of the specimen viewed at normal incidence. Illumination was by unpolarized light from

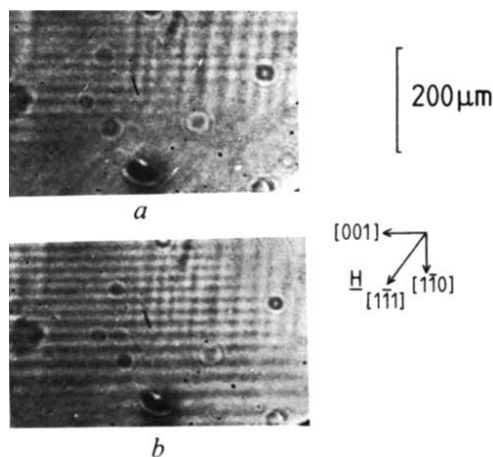


Fig. 1 Optical micrograph using unpolarized light of a (110) surface of a single crystal of terfenol. The striations are due to surface tilts caused by different magnetostrains in adjacent ferromagnetic domains: *a*, zero applied field; *b*, the application of a magnetic field of 3×10^{-2} T in the $[1\bar{1}1]$ direction has resulted in the growth of domains lying parallel to the $[001]$ direction.

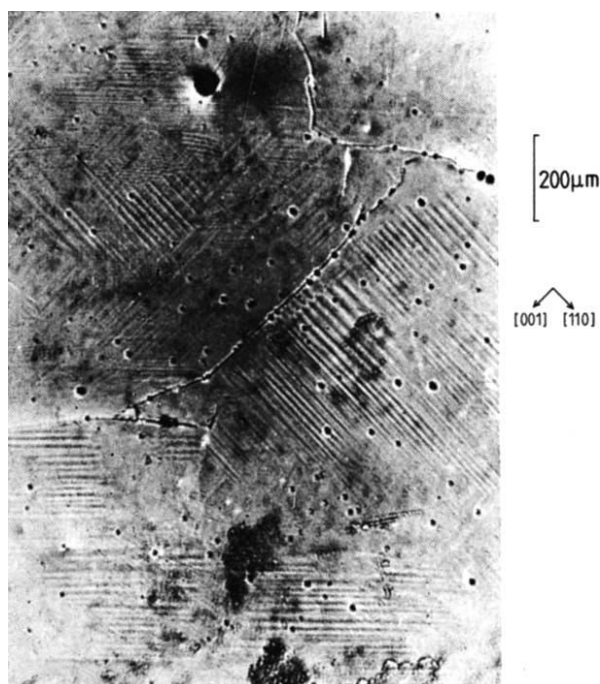


Fig. 2 Optical micrograph of a different part of the crystal of terfenol. Use of polarized light in conjunction with a Nomarski objective shows up the ferromagnetic domains with greater relief.

a conventional incandescent tungsten filament directed roughly normal to the specimen surface. The application of a magnetic field along one of the two easy magnetization directions lying in the surface caused a growth in the number of striations lying parallel to the $[001]$ direction (as seen in Fig. 1*b*). That these images were observable in unpolarized light showed that they did not arise from the Kerr magneto-optic effect⁴. With care these structures could be detected with the naked eye. Figure 2 shows a view of another part of the specimen obtained by using polarized light in conjunction with a Nomarski interference objective. Similar domain configurations have been reported previously in this material when magnetic colloids, magneto-optical methods and synchrotron X-ray topography were used⁷⁻⁹.

We interpret the images presented in Fig. 1 as being due to the tiltings of the specimen surface. From the analysis of the

domain wall types that may be found on the (110) surface in terfenol presented by Clark *et al.*⁸ the domains we observe could be of either the 71° or 109° type.

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Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements

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Research based on ^{15}N stable isotope variations in natural compounds is expanding in scientific fields such as biogeochemistry (isotope fractionation effects measurements¹⁻⁷), metabolic studies^{8,9}, hydrology (research of NO_3^- pollution origin in aquifers¹⁰⁻¹⁴), agronomy (estimate of N_2 symbiotic fixation by legumes¹⁵⁻¹⁷) and oceanography (determination of the source of sedimentary nitrogen¹⁸⁻²¹). However, intercomparison of results obtained in different laboratories is a problem due to the lack of intercalibrated standards. Atmospheric nitrogen has been chosen by many investigators as a standard^{20,22,23} and I present here a simple method for the preparation of atmospheric N_2 as a standard for $\delta^{15}\text{N}$ expression with excellent reproducibility. The results indicate a wide homogeneity in isotopic composition of atmospheric nitrogen which appears to be a reliable standard for ^{15}N natural abundance measurements.

The absolute abundance of the heavy nitrogen isotope, ^{15}N , cannot be assayed with great precision. However, small natural isotope ratio differences between two compounds can be easily measured with high accuracy by using mass spectrometers fitted with double ion-collection and double inlet systems equipped for rapid switching between standard and samples²⁴⁻²⁶.

The most commonly used parameter to describe these differences in natural ^{15}N abundance is $\delta^{15}\text{N}$, the ‰ ^{15}N excess:

$$\delta^{15}\text{N} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1,000$$

where R is the isotopic ratio measured by the mass spectrometer as the ratio of $m/e = 29$ ($^{14}\text{N}^{15}\text{N}^+$) to $m/e = 28$ ($^{14}\text{N}^{14}\text{N}^+$). So, for example, a sample with an isotope ratio 1% higher than

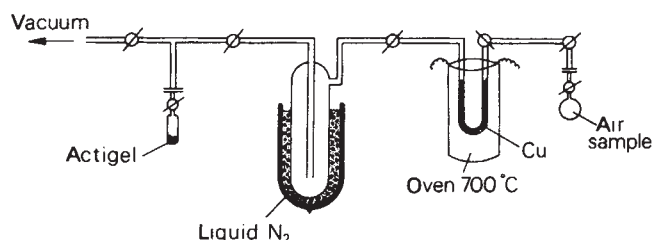


Fig. 1 Apparatus for preparation of atmospheric N_2 for measurement of its isotopic composition.

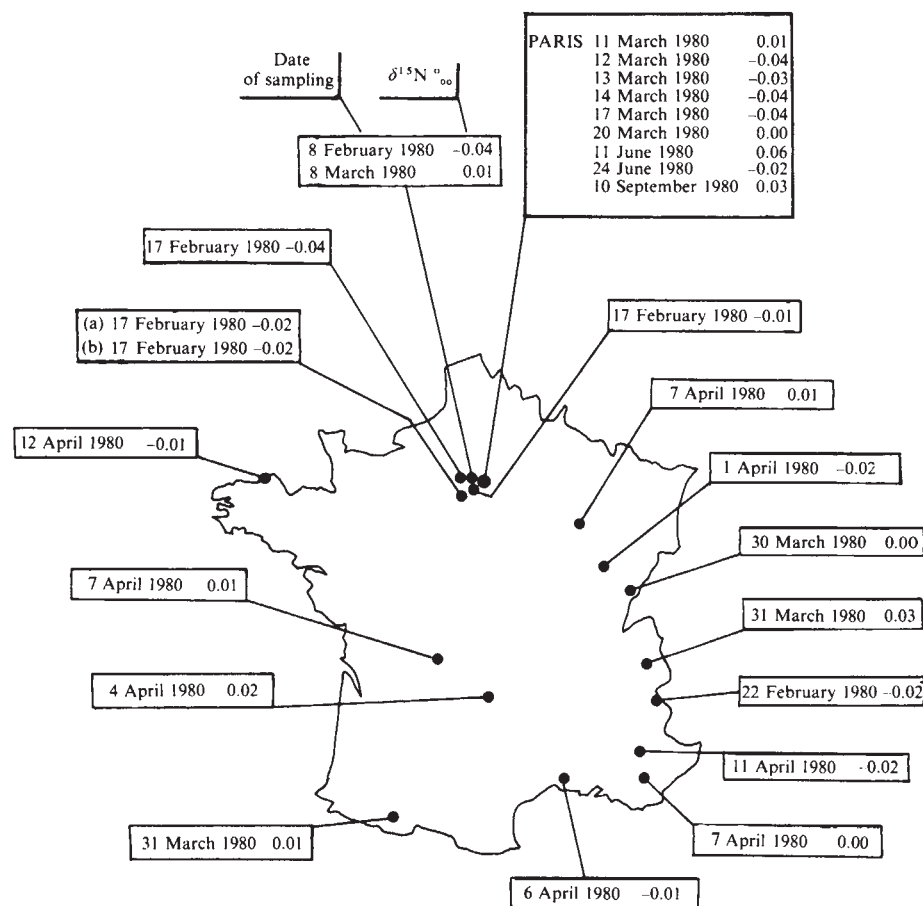


Fig. 2 Atmospheric nitrogen isotopic composition from various localities in France. Data are expressed in $\delta^{15}\text{N}$ units versus one of the samplings arbitrarily considered as standard.

that of the standard against which it is measured would have a $\delta^{15}\text{N}$ value of 10‰. If the standard were atmospheric N_2 (0.3663 atom $^{15}\text{N}\%$), such a sample would have an ^{15}N abundance of 0.3699%.

For independent laboratories to have comparable measurements, a common standard needs to be selected. Such a standard should have perfect homogeneity and chemical and isotopic stability. Moreover, the isotopic composition of the standard must not be very different from that of measured natural samples in order to minimize systematic errors in the mass spectrometer, which increase with the 'isotopic distance' between standard and sample. Thus, a standard which is abundant in nature and of simple chemical form (and coming from a medium where the material is well mixed) is preferred.

An international standard for nitrogen has not yet been defined (as, for example, SMOW for ^{18}O and ^2H in water, or PDB for ^{13}C and ^{18}O in carbonates). Atmospheric nitrogen has been chosen by many investigators primarily through practical experience. The abundance of this element in the atmosphere and its continuous mixing by the wind *a priori* warrant its homogeneity.

A nitrogen gas standard (NBS-14) has been used previously but is no longer available. $(\text{NH}_4)_2\text{SO}_4$ standards (N-1 and N-2) are now on general release from IAEA, Vienna²⁷. The results that we have obtained in our laboratory on these standards by a previously described method²⁸ are presented elsewhere¹⁸. It will be necessary to establish NH_4^+-N , NO_3^--N and organic-N secondary standards, thoroughly checked against atmospheric N_2 , for laboratories to test the reproducibility and fractionation-free procedure in daily preparations of samples other than atmospheric N_2 .

Key steps in the preparation of atmospheric nitrogen for its isotopic analysis²² are deoxygenation and drying of an atmospheric nitrogen sample. Removal of O_2 improves the mass spectrometer stability during measurements, permits higher precision, improves the life span of the filament in the mass-

spectrometer source and removes the possibility of CO production by reaction of carbon in the ion source, with O_2 (ref. 23).

Air sampling is carried out in a vacuum flask. The sample is placed in a simple preparation apparatus similar to the one described in Fig. 1, which operates under a $<10^{-3}$ mm Hg vacuum. An aliquot of this sample is first drawn through pure copper reduced on silver (Carlo Erba product for the CHN elemental analyser), previously heated to 700 °C. The sample is then dried in a liquid nitrogen trap for 5 min and the nitrogen in the sample is subsequently concentrated by adsorption on a molecular sieve (silica gel or active charcoal²⁸) and analysed in a mass spectrometer (Micromass VG 602).

Because the adsorption and desorption of N_2 on silica gel are complete, no isotope enrichment can occur during the procedure. Moreover, we have measured the isotope fractionation factor associated with this procedure by adsorbing only a fraction of pure nitrogen of a well-known isotopic composition. After complete desorption the trapped gas can be analysed. Results are presented in Table 1. The isotopic fractionation factor ($^{14}\text{k}/^{15}\text{k}$), calculated from these experimentations²⁹ is 1.00028 ± 0.00009 . This isotope effect is small enough to be neglected if $>90\%$ of the nitrogen is adsorbed and desorbed.

Table 2 shows data obtained on 10 successive samplings of ultrapure nitrogen from a commercial bottle (N-48; purity, 99.998%). The standard deviation ($\sigma = 0.016$) essentially reflects the reproducibility of the mass spectrometer itself²⁶, rather than variations in the natural ^{15}N abundance of the samples. Moreover, the isotopic composition of pure bottle N_2 remained constant over several years. Hence, this form of nitrogen can be used as a secondary laboratory standard, in spite of its having a different $\delta^{15}\text{N}$ value from that of atmospheric nitrogen (-3.4% versus atmospheric nitrogen for the bottle actually used in our laboratory).

The overall deoxygenation and drying procedure for atmospheric nitrogen described above does not modify the isotopic

Table 1 Measurement of the isotope fractionation factor associated with adsorption of nitrogen on silica gel

N ₂ absorbed on silica gel (%)	Isotopic composition of N ₂ after complete desorption ($\delta^{15}\text{N}\%$)*	Isotopic fractionation factor ($^{14}\text{k}/^{15}\text{k}$)†
19	-0.39	1.00043
40	-0.27	1.00035
50	-0.23	1.00033
50	-0.22	1.00032
60	-0.14	1.00023
60	-0.12	1.00020
70	-0.17	1.00033
80	-0.08	1.00020
80	-0.07	1.00017
Mean		1.00028
Standard deviation		0.00009

* This isotopic composition is expressed versus the isotopic composition of the initial gas used for the experiment.

† Calculated according to ref. 29.

Table 2 $\delta^{15}\text{N}$ values of 10 successive samplings of pure nitrogen ($\text{N}_2 = 99.998\%$) from a commercial bottle

Sampling	$\delta^{15}\text{N}\%$ expressed versus sampling 1
1	—
2	0.01
3	-0.02
4	-0.02
5	0.01
6	0.02
7	-0.02
8	0.00
9	-0.02
10	0.00
Mean	-0.004
Standard deviation	0.016

Data are expressed versus first sampling considered as standard.

Table 3 Isotopic composition ($\delta^{15}\text{N}$) of pure nitrogen ($\text{N}_2 = 99.998\%$) from a commercial bottle with or without treatment (deoxygenation on reduced copper at 700 °C and drying in a liquid nitrogen trap)

	Untreated sample	Treated sample
	0.02	0.04
	0.01	0.00
	0.01	-0.06
	0.01	0.04
	-0.04	-0.04
	-0.01	0.01
	0.01	-0.01
	-0.06	-0.01
Mean	-0.006	-0.004
Standard deviation	0.029	0.035

In the two sets of experimentations, nitrogen is concentrated by adsorption on silica gel before analysis on the mass spectrometer and measured versus a sample of nitrogen from the same commercial bottle without adsorption on silica gel. Both experiments show that no significant isotope effects occur during deoxygenation and trapping on silica gel.

composition of nitrogen as can be shown by comparing untreated with treated samples of ultrapure nitrogen (99.998%) from a commercial bottle (Table 3).

Figure 2 gives data for atmospheric N_2 obtained from various French localities, either in urban areas (Paris metropolitan area) or in the open country (non air-polluted area). These isotopic composition are expressed in $\delta^{15}\text{N}\%$ units versus one of the samples arbitrarily considered as standard. The standard deviation of these isotopic measurements is 0.026 (and only 0.019 if the urban data are omitted).

Samples of whole air submitted to this laboratory from other parts of the world were also measured, the results of which are

shown below (expressed versus the same standard as for data obtained in France):

Vienna (Vienna International Center, Austria): -0.02%
 Saint Louis (Missouri, USA): -0.01%
 Saskatoon (Saskatchewan, Canada): -0.04%
 Baja California (Guerrero Negro, Mexico): +0.04%
 Guadeloupe Island (Caribbean, France): +0.02%

These results are very close to those obtained in France. The quoted standard deviation (0.026) represents an uncertainty of 9×10^{-6} atom $^{15}\text{N}\%$ in the absolute concentration (unknown within this precision) of this rare isotope. Hence, the $\delta^{15}\text{N}$ value of atmospheric N_2 may, for all practical purposes, be considered as universally constant.

This method allows preparation of atmospheric nitrogen for isotopic analysis with excellent reproducibility. Results show perfect and universal homogeneity of isotopic composition of atmospheric nitrogen which appears to be a reliable standard for ^{15}N natural abundance measurements.

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Shallow water bottom topography from radar imagery

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Radar imagery of shallow coastal waters (usually <40 m deep) contain unexplained features¹⁻³ resembling the bottom topography. Since microwave radiation does not penetrate far in sea-water, these features must be the result of hydrodynamic processes coupled to the bottom topography that modulate the amplitude of the short (Bragg resonant) sea-surface waves, the main contributors to the backscattered radar power^{4,5}. Because many different hydrodynamic processes operate in coastal waters, and the precise mechanism which generates the radar images is not fully established, a programme to explore the mechanism(s) was initiated at the US Naval Research Laboratory. Here we give a brief description of the possible mechanisms and results from the pilot experiment conducted in July 1982, 40 nautical miles south-east of Nantucket Island.

US satellite Seasat synthetic aperture radar (SAR) imagery of the ocean depict a wide variety of ocean features ranging

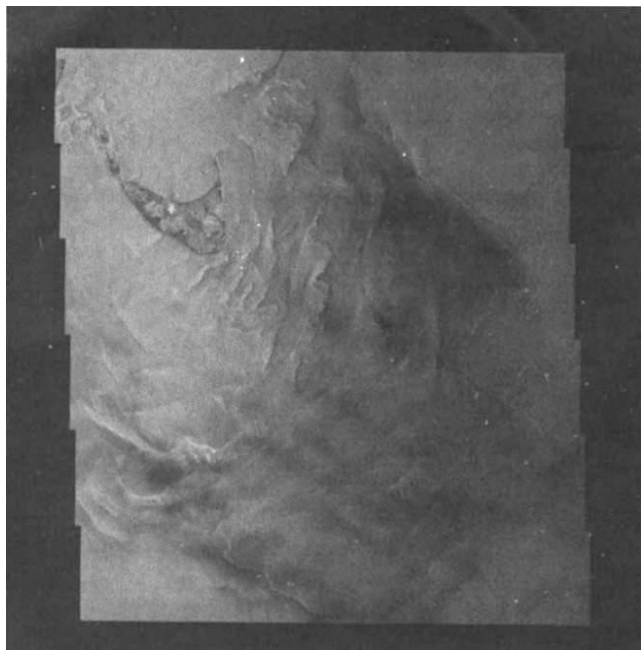


Fig. 1 Seasat SAR image (JPL digital product) of Nantucket Shoals Massachusetts, USA (an area of about $100 \times 100 \text{ km}^2$), taken on 27 August 1978 at 13:34:14 GMT (orbit 880). Phelps Bank is located near the centre of the lower edge of the image.

from mesoscale eddies to internal and surface waves. Furthermore SAR and side-looking airborne, or real-aperture, radar (SLAR) imagery of shallow water contain features that relate almost on a one-to-one basis with the bottom topography¹⁻³. An example from Nantucket Shoals is shown in Fig. 1, and the bottom topography of Nantucket Shoals is given in Fig. 2 for comparison.

The bottom topography features in the radar imagery cannot be the result of direct probing of the sea floor by the electromagnetic radiation, since the microwave fields decay by about two-thirds after having penetrated the water surface by only a few centimetres (1 cm at 1.275 GHz, the frequency of Seasat SAR), while the water depth at the imaged features is of the order of metres to tens of metres. Clearly, these radar imagery features must be the result of hydrodynamic processes, coupled to the bottom topography, that modulate the amplitude of the

short ocean waves with lengths comparable to the radar wavelength (that is, the Bragg resonant waves, 30–40 cm waves for Seasat SAR), known to be the main contributors to the power of the electromagnetic radiation backscattered to the radar sensors⁴. Also it should be recalled that breaking waves contribute to the backscatter power⁵.

Several mechanisms have been suggested for the presence of features in radar imagery which are related to the bottom topography. These include nonlinear refraction, nonuniform shear currents and turbulent wake instabilities affecting the surface waves. However, these proposals have never been formalized or have not been sufficient to explain the features.

More recent work by Zimmerman⁶ and Robinson⁷ provides a basis for understanding the necessary hydrodynamic interactions. Zimmerman showed that "the irregular bottom topography acts as a catalyst for transferring vorticity from the oscillating tidal velocity to the mean (residual) field. A small-scale vorticity field is produced by the Coriolis and frictional torques over an irregular bottom topography and subsequently transferred to the mean field by (nonlinear) advection". Robinson identified three important mechanisms which generate vorticity through the bottom topography which then interacted with the Bragg waves: (1) changes in water depth; (2) lateral shearing of the flow (even when the depth is uniform) due to quadratic bottom friction; and (3) bottom friction when there is a depth-distributed force (even if there is no lateral velocity shear). The residual flow (which persists beyond a tidal cycle) generated by these mechanisms, together with the local tide flowing over the bottom topography, refract, strain and block the surface ocean waves^{8,9}, in particular the Bragg resonant waves. Atmospheric pressure gradients and wind stress also generate residual circulations^{10,11}; the latter is related to bottom topography. Generally the 'along-isobath' residual flow caused by large changes in water depth may result in local wave-breaking of the less energetic ocean waves travelling in the 'cross-isobath' direction that are manifested on the sea surface as semi-permanent 'rip' features which are visible to the naked eye and radar.

The pilot experiment of July 1982, 40 nautical miles south-east of Nantucket Island consisted of two 8-day operations in and around Phelps Bank. The objective of this experiment was a preliminary investigation of current shear-tidal interactions with surface ocean waves. During the experiment radar overflights were made, shipboard radar observations were taken, current and surface wave measurements were made, a hydrographic and bathymetric survey of the area was conducted and meteorological measurements were made throughout each

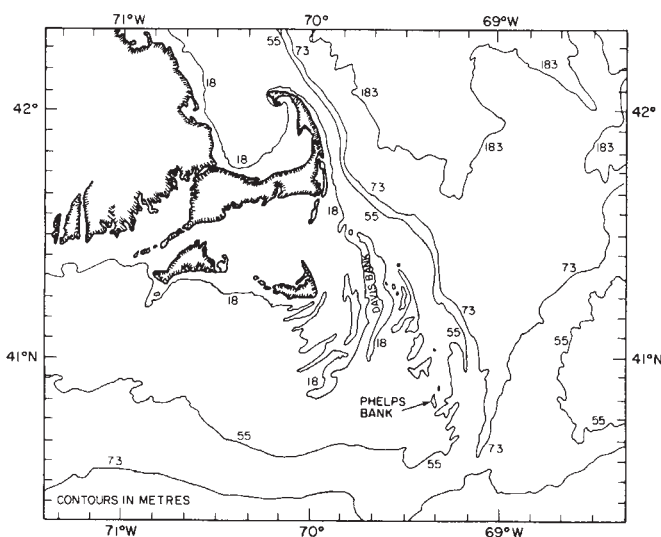


Fig. 2 Bathymetry contours of Nantucket Shoals. The area covered includes the SAR image of Fig. 1.



Fig. 3 Visual evidence of surface expression of bathymetry, taken on 14 July 1982 at 14:50 GMT at Asia Rip, Phelps Bank, Nantucket Shoals, Massachusetts, USA during the NRL remote sensing experiment. Asia Rip is located approximately at $40^{\circ}47'30'' \text{ N}$, $69^{\circ}21' \text{ W}$. The bottom is sloping up gradually of the order 6×10^{-3} to 21 m depth at the Rip and then abruptly deepens with a slope of 0.121 to 38 m. Six-second waves are coming towards the ship and the main tidal flow is about 1 m s^{-1} towards the Rip. The wind is 4.5 m s^{-1} , also towards the Rip.

operation. Figure 3 was obtained on 14 July 1982, at Asia Rip in the Nantucket Shoals. The rough water away from the ship is probably a rip current caused by the mechanisms cited above. Radar (SLAR) overflights of the area at X-band (9.375 GHz) also show large (>20 db) increases in backscatter power over the rough water indicating strong modulation of the Bragg resonant waves.

SLAR imagery of the ocean is only a function of the radar cross-section of the ocean which is proportional to the energy of the short Bragg resonant waves⁴, while on the other hand SAR imagery depends on both the radar cross-section of the ocean and the phase distortion caused by the motion of the radially traveling Bragg resonant waves which are advected by the fluid motion of longer ocean waves and currents¹²⁻¹⁴. However, in shallow coastal waters the currents are mostly tidal in nature with residual circulation related to the bottom topography of the area. Therefore, it is not surprising that radar imagery of shallow coastal waters contains features related to the topographic (Fig. 1) characteristics of the sea floor.

An estimate of the range of validity of the proposed mechanisms may be obtained from previous work^{7,10}; they are proportional to tidal current and should predominate for winds <10 m s⁻¹. The magnitude of residual flow depends on the change in depth-to-actual depth ratio, the length of the vorticity-generating region, the bottom slope and the transverse length scale associated with tidal velocity shear. Preliminary results from the Phelps Bank Experiment are in basic agreement with our ideas.

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Palynological evidence for Miocene climatic cooling in eastern Iceland about 9.8 Myr ago

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The processes which led to the Pleistocene glaciations in the Northern Hemisphere are presently known only from fragmentary evidence of major Tertiary climatic cooling events^{1,2}. Although there is good evidence³ for glacial intervals in Alaska 10-9 Myr, most terrestrial records^{4,5} which suggest a corresponding high-latitude climatic cooling in the north-east Atlantic are poorly dated, and North Atlantic palaeoceanographic records contain large erosional hiatuses^{2,6}. We have obtained new palynological data from eastern Iceland, from clastic units between lava formations dated by K-Ar and palaeomagnetic methods^{7,8}. We now report that these data record a floral succession which suggests that a temperature decrease of ~10 °C occurred in Iceland between 10 and 9.5 Myr.

The stratigraphical setting for our studies is the Tertiary lava pile in Iceland which contains plant-bearing clastic beds on either side of the present active volcanic zone⁹. The Tertiary flora is best known from clastic beds in western Iceland, from which a history of climatic cooling from late Miocene (8 Myr) to Pliocene (~5.5 Myr) has been reconstructed^{9,10}. The chronology and palaeoclimatic significance of the eastern Icelandic Tertiary flora, however, has been less certain. Here, the oldest floristic horizon¹¹, at Gerpir, has K-Ar dates¹² of 13-12 Myr. This flora is dominated by Pinaceae and Fagaceae-type pollen¹¹, suggesting a cooler climate than that of the oldest flora (14-13 Myr) in western Iceland, which is dominated by Taxodiaceae and thermophilic hardwoods⁹. In eastern Iceland, the Gerpir horizon is overlain by about 3 km of lava flows, above which the flora-bearing Holmatindur clastic formation is found in the Reydarfjörður area, ~30 km west of Gerpir (Fig. 1). The Holmatindur flora has variously been assigned an age of late Eocene¹¹, late Miocene¹² or Plio-Pleistocene¹³, based on different opinions concerning the correlation of its mixed floral elements (thermophilic and boreal) with well-dated western Icelandic floras.

These early studies of the Holmatindur flora were made from samples of lignite beds from Tungufell Mt (site 1, Fig. 1) and the north side of Holmatindur Mt (site 2, Fig. 2). In 1957, Meyer and Pirrit¹³ studied a 4-m thick seam of tuff with fossiliferous layers at about 560 m on the north flank of Tungufell Mt. The salient features of this flora are shown in Table 1. Meyer and Pirrit¹³ concluded that these assemblages represent the youngest Icelandic flora before elimination of the Taxodiaceae and thermophilic hardwoods (including *Nyssa* and *Carya*) by a presumed 'ice age' glaciation between the late Miocene and early Pleistocene. In 1974, Bratseva¹² described the palynoflora of lignite partings in a 7-m section of the Holmatindur tuff on north Holmatindur Mt, at ~750 m. Seven samples were analysed but only a general account of the flora was given (see Table 1). Akhmetiev et al.¹² correlated this flora with that of the Middle-Upper Miocene sediments of Germany and Denmark, but they noted that the Icelandic flora contained a lower percentage of thermophilic taxa and fewer species.

In 1978, a continuous core was drilled through the Tertiary basalt lavas near the head of Reydarfjörður, and studies were made of stratigraphical sections^{7,14} around the fjord to correlate the core with the K-Ar dated stratigraphical sections of Watkins and Walker¹⁵, located ~10 km north of Reydarfjörður. The lithological succession at Reydarfjörður has now been divided into several formations (Fig. 1) consisting mainly of lavas but also including about 10% clastic material^{7,16}. Individual clastic units that intercalate the basalt lavas are usually part of wide-spread sedimentary cycles, some of which can be traced in the strike direction (N 2° W) for about 70-80 km. In the dip direction (W) the clastic units are exposed for about 15 km and they do not thin out before their submergence at sea level.

The Holmatindur Tuff Formation is one of the best known eastern Icelandic clastic cycles^{7,16}. In the Reydarfjörður area, this clastic cycle has been traced from Holmatindur Mt to Tungufell in the north¹² and southward to Eyrafjall¹⁴ (Fig. 1). The clastic units in four profiles of the cliffs at Reydarfjörður recently were correlated^{7,8} with the core and the sections to the north¹⁵, using lithological, K-Ar and palaeomagnetic methods. It is now clear that the lava formations enveloping the Holmatindur tuff formation are of normal magnetic polarity and form part of magnetic anomaly 5 (epoch 9). The Holmatindur Tuff Formation has a late Miocene age of ~9.8 Myr; it is definitely younger than 10.3 Myr and older than 9.5 Myr (refs 7, 8).

For our palynological studies, lignite horizons were sampled in the Holmatindur tuff at two of the Reydarfjörður cliff profiles. Five samples were obtained from the south side of Holmatindur Mt at an elevation of 725-800 m (Fig. 1, site 3); six samples were taken from the Holmatindur clastic unit in the Eyrafjall cliff profile, between 650 and 670 m (Fig. 1, site 4). The stratigraphical relationship and lithological characteristics of the samples are listed in Table 2. Samples (10-20 g) were processed

Table 1 Pollen and spores in samples from the Holmatindur Tuff Formation

	Sample														
Taxon	(1)	(2)	RF -C	76/75 -F	-H	RF 79	RF 80		6b	15	B4 18	20	23	28	
<i>Laevigatosporites</i> (Th)	c	a	2	2	2	4			6	6	2				
<i>Lycopodium</i> (M)		a	7	4			8		41	2		4	4		
<i>Osmunda</i>	a	a	47	38		35			7	1	122		20	4	
<i>Pteridium</i>			2	2	2		2		3		52	4	1		
<i>Sphagnum</i> (M)	o				2	4	2			2			2		
Conifers															
<i>Larix</i> (B)	o		5	2	8				49		22		5	2	
<i>Picea grandivescipites</i> (B)			5	106	54	10			21	4	34	9	20	20	
<i>Picea</i> (B)		r		8	22	28			3	2			3	16	
<i>Pinus</i> (B)		r	2	19		7			24	1	4	3	3		
<i>Pseudotsuga</i> (Th)									7						
<i>Sequoia</i> (T)	c								3						
<i>Taxodium</i> (T)	c		5						45						
<i>Tsuga</i> (Th)									6		2				
Angiosperms: trees															
<i>Alnus verus</i> (A)	a	c			6	60			2		86	3		4	
<i>Betula cf. claripites</i> (B/Th)	c	c	5	2		24					1	1	2		
<i>Acer</i> (Th)		r	7	14	2				25	1	8				
<i>Carpinus/Ostrya</i> (Th)											16				
<i>Carya</i> (Th)	r								2		2				
<i>Celtis</i> (Th)		r									4	10			
<i>Corylus</i> (Th)	c									2	4		1		
<i>Fagus</i> (Th)	c	c	5						39		8				
<i>Juglans</i> (Th)	c	c	3						7		15		1		
<i>Nyssa</i> (Th)	r														
<i>Pterocarya</i> (Th)		r									23				
<i>Quercus</i> (Th)	c	o							8		14				
<i>Salix appiculata</i>									16						
<i>Ulmus</i> (Th)	c	r	5						6		24				
Angiosperms: shrub/herb															
<i>Alnus viridus</i> (A)		c		20	6	48	4						1	26	
<i>Betula</i> sect. <i>Nanae</i> (A)		c			4	4							6	12	
Caprifoliaceae		r													
Cyperaceae (A)					4	4			6				1	4	
Ericaceae (A)	r	r	2	1								2	1		
Gramineae (A)					4					1	1			4	
Other taxa									24	1	28	2	6	8	
Total	a	a	102	218	116	228	16		350	23	472	38	77	100	

a, Abundant; c, common; o, occasional; r, rare. (1) Tungufell data of Meyer and Pirrit¹³; (2) northern Holmatindur data of Bratseva¹². RF 76/75, 79, 80 and B4 are samples from the present study (see Table 2), with data shown as counts of pollen and spores. Letters in parentheses indicate vegetation units of Fig. 2. (A) Alder scrub; (B) boreal forest; (M) moss/clubmoss; (T) Taxodiaceae; (Th) thermophilic hardwoods.

using dilute HCl and 52% HF for removal of clastics, and ZnBr₂ flotation for palynomorph concentration. Most of the palynomorphs found are listed in Table 1. Duplicates of two processed samples (B4-18 and B4-20) were counted by Dr Glenn Rouse, University of British Columbia, whose taxonomy¹⁷ is followed here. The salient features of the palaeoecological data for the Holmatindur Tuff Formation are summarized in Fig. 2 and are described as follows.

At Site 3, three samples (RF 76/75 C, F, H) were taken from lignitic layers about 4.5 m above the base of the Holmatindur Tuff Formation. The lowest sample (C in Fig. 2) is a grey argillite with fragments of large dicotyledon leaves and impressions of *Osmunda* fronds. It has a relatively sparse spore flora dominated by *Osmunda* (*O. claytonites* and *O. cinnamomites*), and a pollen flora dominated by *Acer*, with smaller amounts of *Fagus*, *Picea grandivescipites*, *Larix* and *Taxodium*. This palynoflora is similar to that described by Bratseva¹² for Site 2 but it contains no *Alnus* and has fewer thermophilic hardwood species. Sample F has a palynoflora dominated by *Picea grandivescipites* and a small *Pinus* sp., with common *Osmunda* spores but rare thermophilic hardwoods, *Acer* being the only representative. Sample H is also dominated by *Picea* but it contains no *Osmunda*. *Alnus* (both *A. verus* and *A. viridus*-type) appear in this sample although other moisture-demanding species are not present. Overall, the sequence from C to H suggests a major climatic cooling, ranging from a warm

temperate climate with mixed hardwood-conifer forest to a cool, then cold boreal climate.

Sample 79 (Fig. 2) was obtained from mudstone in a 4.5-m thick clastic unit about 40 m above RF 76/75. Sample 79 contains abundant palynomorphs which are dominated by *Alnus*, with a low diversity of other species (mainly *Betula*, *Picea* and *O. cinnamomites*). This palynoflora contains no thermophilic tree pollen species. Sample 80 is from a 1-m thick clastic bed ~10 m above sample 79. It contains a sparse palynoflora of *Lycopodium*, *Pteridium* and *Alnus*. This flora indicates a very cold (subarctic) climate or a depauperate post-fire vegetation.

At Site 4, the lowest sample, 6b (Fig. 2), is a fine sandstone containing a rich palynoflora dominated by *Larix*, *Taxodium* and *Fagus*. Species diversity is high and thermophilic trees are fairly well represented, including *Sequoia*, *Ulmus*, *Juglans* and *Carya*. Sample 15 (Fig. 2) is a coarser sandstone containing a sparse palynoflora dominated by fern and moss spores, suggesting a turbulent fluvial environment or some other temporary ecological change. The overlying grey argillite (Sample 18) has a rich flora which is almost identical to that described for site 2 but has a slightly higher percentage of *Picea* and *Pinus* and lower percentage of *Fagus*. According to Glenn Rouse (personal communication), this palynoflora is very similar to that of the upper Skokun Formation¹⁷ of the Queen Charlotte Islands, western Canada. Sample 20, a grey siltstone, has a

Fig. 1 Geological map of western Reydarfjörður area, showing stratigraphical marker formations, known extent of the Holmatindur Tuff Formation and the locations of palynology sample sites: 1, Tungufell; 2, northern Holmatindur; 3, southern Holmatindur, section RF; 4, Eyrafjall, section B4. Holmatindur Mt. IRDP, the Iceland Research Drilling Project core site. Inset shows the location of the Reydarfjörður research area in eastern Iceland. (Modified after refs 7, 16.)

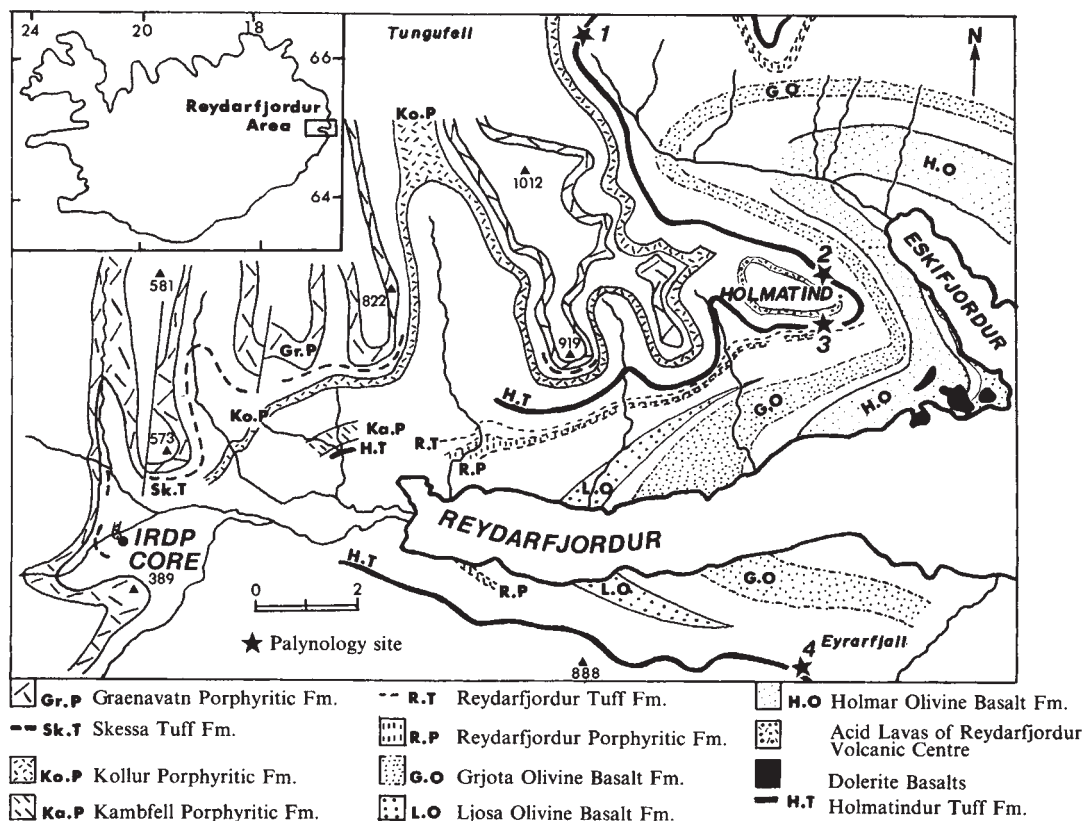


Table 2 Stratigraphical position and lithological features of palynology samples listed in Table 1

Site	Location	Ref.	Sample no.	Altitude (m)	Lithology
1	Tungufell	13	(1)	560–630	Tuff with carbonaceous or lignite bands
2	Northern Holmatindur	12	(2)	750–757	Coaly argillite grading up to tuffaceous sandstone
3	Southern Holmatindur, Section RF		RF76/75-C	729.6	Grey argillite with leaf impressions
	Present study		RF76/75-F	730.5	Fine white sandstone with lignite partings and leaf imprints
			RF76/75-H	731.5	Black siltstone with leaf impressions
			RF79/78	790	Brownish-grey argillite
			RF80/79	800	Carbonaceous argillite
4	Eyrafjall, section B4		HTT-B4-6b	651–658	Grey sandstone with thin lignite partings
	Present study		HTT-B4-18	622.6–662.9	Carbonaceous grey sandstone
			HTT-B4-20	664–666	Grey siltstone with plant fragments
			HTT-B4-23	666–669.6	Brownish-grey sandy siltstone with thin lignite partings
			HTT-B4-28	671.5–671.8	Grey sandstone

sparse palynoflora dominated by *Picea* and *Celtis*, with a low diversity of shrub and fern species. Samples 23 and 28 are sandier sediments from ~2 and 3 m above sample 20, respectively. They contain palynofloras dominated by *Picea*, with a strong representation of cold-tolerant shrubs, including *Betula* sect. *Nanae* and *Alnus viridis*. By analogy with modern floras¹⁸, the Eyrafjall assemblages denote a succession of vegetation types, ranging from a basal one resembling North Carolina deciduous forest (mean annual temperature 13–15 °C), followed by a Great Lakes-type deciduous forest (7 °C), and terminating in boreal forest or woodland similar to that of the Canadian Atlantic region (3–5 °C). The similarity between the Eyrafjall and Holmatindur floral successions (Fig. 2) indicates that the cliff sections south and north of Reydarfjörður record essentially the same floristic changes despite the differences between their lithofacies.

Previous regional studies of the Icelandic Tertiary flora^{5,9} have concluded that floras older than 8–9 Myr were warm temperate deciduous forests and that boreal forest or shrub vegetation indicates a flora of latest Miocene (<5.5 Myr) or Plio-Pleistocene age. The data we have obtained, however, suggest that the Holmatindur Tuff Formation records a floral

spectrum ranging up-section from a strongly thermophilic swamp forest (*Taxodium*-hardwoods) to cool temperate deciduous-boreal forest, then microthermal spruce forest, and finally, subarctic alder woodland. This suggests the occurrence of a major climatic cooling event during the time interval of 10.3–9.5 Myr. However, other explanations should be considered, for example: (1) latitudinal drift; (2) altitudinal change; (3) lithofacies change; (4) fire succession; and (5) volcanism.

(1) The range in proxy-climatic indicators cannot be ascribed to northward drift of Iceland because east-west spreading has dominated for the past 10 Myr (ref. 19). (2) Palaeoaltitudes of the sample sites show a maximum elevation difference of ~150 m over a distance of 13 km, which is unlikely to account for a wide range of climatic indicators. (3) The lithological data in Fig. 2 show that there is no consistent correlation between sediment texture and palynomorphic composition. (4) It is possible that fire-succession accounts for part of the vegetation change; however, samples B4-15, -23 and RF 80 all contain abundant charcoal fragments, yet they have very different pollen spectra. (5) Post-volcanism vegetation succession is an unlikely explanation because the Reydarfjörður clastic units represent quiescent intervals of 16,000–50,000 yr duration

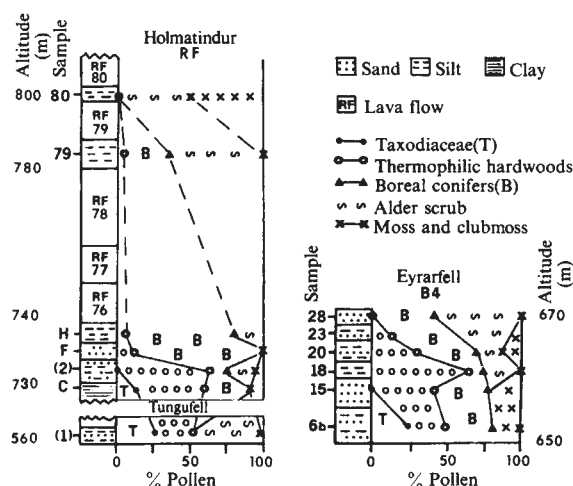


Fig. 2 Summary of palynological and lithological data from the Holmatindur clastic units north (Holmatindur) and south (Eyrarfjall) of Reydarfjörður. Sample numbers refer to Tables 1 and 2. Vertical axes indicate approximate altitudes (note reduced scale from 740 to 780 m). Pollen data are plotted as cumulative percentages, where 100% is the total number of pollen and spores, excluding *Osmunda* which is often locally over-represented. The Tungufell (1) and northern Holmatindur (2) samples are placed in their inferred relative stratigraphical positions.

which is much longer than known rates of reforestation on volcanic islands²⁰.

We therefore postulate that the palynoflora of the Holmatindur Tuff Formation records a major cooling event that predates the oldest Icelandic tillites (4.8–6 Myr)²¹ and postdates the high-latitude Oligocene cool episode recently described from Mackenzie Delta palynofloras¹. The Holmatindur flora is considerably older than the cool temperate Hredavatn flora (~7 Myr)^{5,9} yet the upper Holmatindur palynoflora is more similar to the Pliocene Tjornes flora⁵ than the Hredavatn flora. The age of the inferred Holmatindur climatic cooling corresponds closely to that of the oldest Alaskan tillites³ and suggests that the events which led to the Alaskan glaciation were of widespread occurrence at high latitudes in the Northern Hemisphere. Confirmation of an early late Miocene climatic cooling in Iceland would provide important evidence in support of arguments² for substantial Northern Hemisphere ice sheet growth before 5 Myr and it may clarify the controversy about the timing of increased Arctic water flow over the Iceland–Faroe ridge during the Miocene^{6,19}. Finally, we stress the stratigraphical importance of verifying the existence of a major climatic cooling event in Iceland during the early late Miocene because palynological correlation of Icelandic lignite beds⁵ is often based entirely on the presence or absence of thermophilic pollen elements, with the assumption that a warm temperate flora persisted throughout Iceland until at least 8 Myr.

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Sulphur in sediments chronicles past changes in lake acidification

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Sulphur pollution has closely been linked to the problems of acid precipitation and lake acidification¹. Although variations in sulphur isotopes can in principle be used to fingerprint the sources of sulphur in the lacustrine environment (see refs 2–5), no attempt has so far been made to apply the technique in the study of pollutant sulphur in lakes in an historical sense. We report here the concentrations and isotopic composition of sulphur in sediments of several softwater lakes of northern Ontario. An objective of the study has been to ascertain whether the accumulation of sulphur in the sediments is related to the flux of pollutant sulphur into the lakes.

The lakes studied are located in two contrasting areas with regards to sulphur pollution. The first group of lakes is located near Sudbury and are expected to derive most of their sulphur from the big nickel–copper smelter nearby^{4,6}. This point source has a fairly constant isotopic signature⁶ and the sedimentary records of sulphur pollution in these lakes should be related to the local history of smelting activities. Many of the lakes have become acidic. On the other hand, the second group of lakes are located in the more remote Algonquin Provincial Park with no known major local sources of sulphur. The historical records and isotopic profiles in the sediments presumably would reflect the flux of long-range transported sulphur into the lake basin.

The procedures used in obtaining the sediment samples and in the ²¹⁰Pb dating of the core samples have been described elsewhere⁷. The sulphur in each sample was converted to the sulphate by fusion with a mixture of sodium and potassium nitrates^{8,9} and then determined gravimetrically as barium sulphate. Subsequently, the BaSO₄ was decomposed thermally to liberate the SO₂ (ref. 10) and the ³⁴S/³²S ratios of the SO₂ samples measured on a dual-collector isotope ratio mass spectrometer (VG Micromass, model 602C). The isotope ratio results are given in the usual $\delta^{34}\text{S}$ notation:

$$\delta^{34}\text{S} (\text{‰}) = (R_A/R_B - 1) \times 1,000$$

where R_A and R_B refer to the ³⁴S:³²S ratios of the sample and the reference Canyon Diablo troilite, respectively. The reproducibility of the measurements are $\pm 10\%$ for the total sulphur and $\pm 0.3\%$ for $\delta^{34}\text{S}$, based on replicate analyses of several sediment aliquots.

The profiles of total sulphur and $\delta^{34}\text{S}$ in representative lake sediments are shown in Fig. 1; the same basic type of profiles have been found in the other lakes we have also analysed. A prominent feature of the profiles is the spectacular enrichment of sulphur (referred to here as the excess sulphur) in the top sediment layers. The ratios of the total-sulphur in the surficial (0–1 cm layer) sediments to the average concentration in the deeper or precolonial horizons (defined here as an enrichment factor, EF) are summarized in Table 1. It is significant that the two lakes (Ramsey and MacFarlane) closest to Sudbury have the highest EF values.

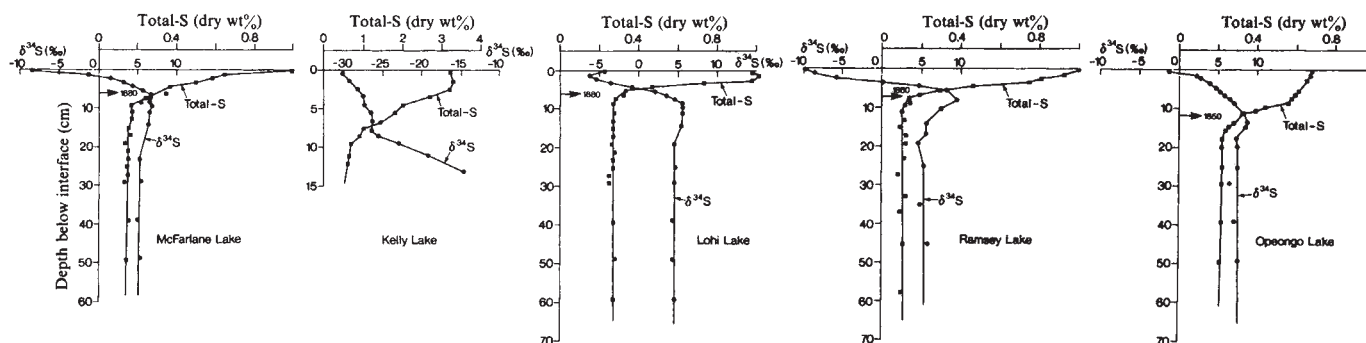


Fig. 1 Profiles of total sulphur and $\delta^{34}\text{S}$ in sediments of representative softwater lakes of northern Ontario.

For the lakes around Sudbury, the dramatic increase in the accumulation of sulphur in the sediments dates to about 1890 (see ref. 7 for geochronological details); in other words, coincides with the beginning of smelting of the copper-nickel ores in Sudbury⁷. By contrast, the pronounced increase in the accumulation of sulphur in Windy Lake sediments, located ~20 km from Sudbury, dates only to ~45 yr BP (dating details in ref. 7), and hence coincides with the installation of a much taller 170 m stack at Copper Cliff in 1923. It is relevant that the plumes from the 381 m stack at Sudbury can be traced for distances of up to 100 km (ref. 4). The sediment samples from Algonquin Park are being dated (H. K. T. Wong and J.O.N., unpublished results); the preliminary data show that the accumulation of the excess sulphur began around 1860–70. In other words, the flux of excess sulphur (or 'acid rains') into lakes in the park coincides with the beginning of extensive industrialization of the Great Lakes basin. The sensitivity of the sediments to the influx of sulphur from a local or distant sources is clearly demonstrated. The historical relationships described above make a strong case that the deposition of the excess sulphur reflects the increasing flux of pollutant sulphur (or 'acid rains') into the lakes. It is significant that all the three lakes (Ramsey, Richard and MacFarlane) located <7 km from Sudbury have identical total-sulphur and $\delta^{34}\text{S}$ values (Fig. 1). Also the excess sulphur contents of the lake sediments near Sudbury are much higher than those located in Algonquin Park (see Fig. 1).

The changes in the $\delta^{34}\text{S}$ profiles closely parallel the input of excess sulphur into the lake sediments (Fig. 1). Without exception, the most recently deposited sulphur is lighter (has lower $\delta^{34}\text{S}$ values) than the sulphur in the older sediments. More importantly, the shift in the $\delta^{34}\text{S}$ coincides with the onset of excess sulphur influx into the sediments (Fig. 1). The excess sulphur in surficial sediments near Sudbury is much lighter (average $\delta^{34}\text{S}$ value, about -8‰) than that in Algonquin Park sediments of comparable age (mean $\delta^{34}\text{S}$ value of only -0.5‰). Such a difference in $\delta^{34}\text{S}$ values of surficial sediments can be viewed in relation to the fact that the $\delta^{34}\text{S}$ for the Sudbury ores is nearly zero¹¹ and the $\delta^{34}\text{S}$ value for the SO_2 from the smelter stack at Copper Cliff is +1 to +2‰ (ref. 4). On the other hand, the $\delta^{34}\text{S}$ value for rainfall in the Great Lakes basin is +1 to +9‰ (ref. 12), while the value for ambient air generally varies from about +5 to +10‰ (refs 13–16). Perhaps, the difference in the isotopic signature of the local and regional sources of sulphur is reflected in the heavier excess sulphur in the Opeongo sediments. The highly contaminated sediments of Kelly Lake (a recipient of both domestic and industrial effluents) contain up to 3.3% sulphur which is highly depleted in ^{34}S ; the $\delta^{34}\text{S}$ varies from -20 to -30‰ (Fig. 2). The isotopic data thus point to close relationship between the influx of pollutant sulphur and the accumulation of excess sulphur in the lake sediments. The same phenomenon has been observed in the Great Lakes³.

A progressive reduction of the sulphate to sulphide in the water column and the precipitation of the resultant ^{34}S -depleted sulphide in the sediments could conceivably generate the total-sulphur and $\delta^{34}\text{S}$ profiles observed. This seems unlikely, however, since few of these lakes develop anoxic hypolimnion

necessary for sulphate reduction (see ref. 17). Post-depositional migration of sulphur in the sediment should result in the net loss of ^{32}S from the sediment and the expected profile should thus be the exact opposite of what has actually been observed (Fig. 1). Furthermore, our preliminary results (Y. K. Soon and J.O.N., unpublished) show that acid-volatile sulphides are very much a minor component and that organic sulphur generally accounts for 50–70% of the sulphur in these sediments; we know of no mechanism to explain the large fractionation of sulphur isotopes in the organic material. The variations in the total sulphur contents of the sediments (Fig. 1) presumably is related to the differences in the organic carbon concentrations (Table 1). The fact that identical total-sulphur and $\delta^{34}\text{S}$ profiles are found in sediments with widely differing organic carbon concentrations tends to downgrade any suggestion of biogenic controls on the accumulation of excess sulphur in the sediments. The total-sulphur or the $\delta^{34}\text{S}$ profiles observed leave little doubt that the recent changes in the sulphur contents of the sediments is related to the increasing flux of pollutant sulphur into the lakes.

Although sediments generally constitute an important sink for sulphur in limnetic environments, few studies have actually related the sedimentary sulphur to lake acidification. The total-sulphur and $\delta^{34}\text{S}$ profiles which Cook⁵ found in the Experimental Lakes Area of northern Ontario are very similar to those reported here. In their study of sulphur in two softwater lakes in the Adirondack Mountains, New York, Mitchell *et al.*¹⁸ used very coarse sampling intervals (every 5 cm) which would

Table 1 Enrichment factors for sulphur and the organic carbon contents of surficial sediments

Lake	Location	Enrichment factor	Organic carbon concentration (wt %)
Windy	32 km NW of smelter (Sudbury basin)	1.9	15
Ramsey	7.2 km E of smelter (Sudbury basin)	9.1	4.6
Wavy	19 km S of smelter (Sudbury basin)	1.0	12
Kelly	3 km S of smelter (Sudbury basin)	—	8.9
Macfarlane	10 km SE of smelter (Sudbury basin)	6.5	6.2
Lohi	10 km S of smelter (Sudbury basin)	3.8	9.6
Nelson	29 km N of smelter (Sudbury basin)	4.0	14
Richard	11 km SE of smelter (Sudbury basin)	4.6	8.6
Vermillion	26 km W of smelter (Sudbury basin)	1.9	2.3
Opeongo	Algonquin Provincial Park	3.4	10
Kioskokwi	Algonquin Provincial Park	2.3	9.4

have obfuscated any recent changes in total-sulphur concentrations. Our data for lakes in northern Ontario support the recent suggestion¹⁹ that sedimentary sulphur can be used as a time tracer for acid rain deposition in the eastern United States. The sediment samples are now being fractionated into acid-volatile sulphur, elemental sulphur, organic sulphur and pyrite sulphur (Y. K. Soon and J.O.N., unpublished results), and the isotopic technique will be used to assess the principal pathways of pollutant sulphur flux into the sediments. It is expected that such data will make it possible to quantify the historical changes in the flux of acidic sulphur compounds into the lakes. There are currently few reliable indices of past changes in the buffering capacities of lakes which can be attributed to acidic precipitation.

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Dynamic effects from mantle phase transitions on true polar wander during ice ages

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The relaxation spectrum of a stratified viscoelastic mantle with phase transitions contains a class of extremely long decaying modes with relaxation times $0(10^6 \text{ yr})$, exceeding the characteristic time scale of an individual glacial cycle. Consequently, the net motion of the rotation pole produced by the cyclic loading and unloading of ice sheets would display a nonequilibrium behaviour in the initial stages of an ice age epoch. We present here calculations of the net polar speed as a function of time for the late Cenozoic ice ages. These results show an initial transient net velocity of $0(1^\circ \text{ Myr}^{-1})$ which, after a few million years, decays to a steady value of $0(0.1^\circ \text{ Myr}^{-1})$. We propose that the length of a typical ice age period around $0(10^7 \text{ yr})$, is controlled by the time scale required for the steady-state polar wander to drift sufficiently far such that the necessary conditions for the Milankovitch mechanism to operate can no longer be maintained.

The phenomenon of true polar wander (TPW) since the late Cretaceous^{1–7} may have a bearing on the diverse areas of mantle convection³ and the evolution of ice ages², and can also influence the duration of ice ages from earlier geological epochs, such as the Silurian which took place some 400 Myr ago.

In general, TPW is caused by changes in the Earth's moment of inertia as a result of a redistribution of mass in its interior

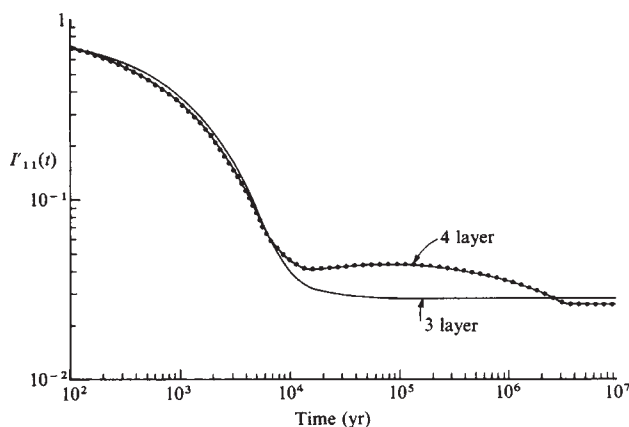


Fig. 1 Temporal evolution of the equatorial moment of inertia from a surface point mass load. $I_{11}(t)$ has been non-dimensionalized with respect to the change of moment of inertia caused by the load itself. The density profile in the mantle is smooth for the three-layer model, whereas in the four-layer model a density jump of 0.4 g cm^{-3} is present at 670 km depth. The mantle viscosity is uniform in both models at 10^{22} P .

and of the accompanying shape distortion of the planet. For time scales greater than $0(10^3 \text{ yr})$ the most likely candidate for the excitation of TPW is motion of density inhomogeneities in the mantle^{8,9}. We now consider the part played by mantle phase transitions in driving transient mantle flows, which serve to excite secular polar movements^{1,2}.

From previous work² based on calculations of a three-layer Earth model, which consists of an elastic lithosphere, a viscoelastic mantle and an inviscid core, we found that the late Cenozoic ice ages¹⁰ are capable of producing an averaged polar wander of $0(1^\circ \text{ Myr}^{-1})$ for a mean mantle viscosity of 10^{22} P . This result suggests that TPW furnishes a mechanism which could eventually terminate ice ages within a few million years². The argument stems from the changes induced in the overall land-sea configuration with respect to the spin axis from TPW, which perturbs the current set of surface boundary conditions, which are favourable for cyclic glaciation to occur by the Milankovitch theory^{11,12}. However, a realistic Earth model requires that the effects of density jumps due to mantle phase transitions, which involve a change of the crystal structure at the same chemical composition, must also be considered. The density profile is taken to be adiabatic everywhere except at the depths of phase change, which in the interior of mantle convection cells are neither depressed nor elevated¹³. Accordingly, the normal stress is continuous across the density discontinuity at 670 km depth. Recent studies^{14,15} of four-layer viscoelastic models which takes into account the phase changes have revealed the presence of an extra mode, M1, with a relaxation time $> 10^6 \text{ yr}$. As the time scale of this phase transition mode exceeds those of the other modes by $0(10^2)$ (refs 14, 15) and that of the glacial cycle in the Quaternary by $0(10)$, it becomes essential to assess the dynamic effects produced by this singular mode on the average speed of polar wander. This model consists of an inviscid core, an elastic lithosphere, a two-layer viscoelastic mantle, with a density jump at the appropriate depth. The density and shear modulus of each of the mantle layers is obtained by averaging of realistic seismic models¹⁶. The mean density of the core is taken to be $10,930 \text{ kg m}^{-3}$. The thickness of the elastic lithosphere is assumed to be 100 km. The parameters, which are varied in this study, are η_1 , the lower mantle viscosity, η_2 the upper mantle viscosity. In Fig. 1 we show the effects of a mantle phase change on altering the time scales of isostatic readjustment by comparing the temporal development of the perturbed moment

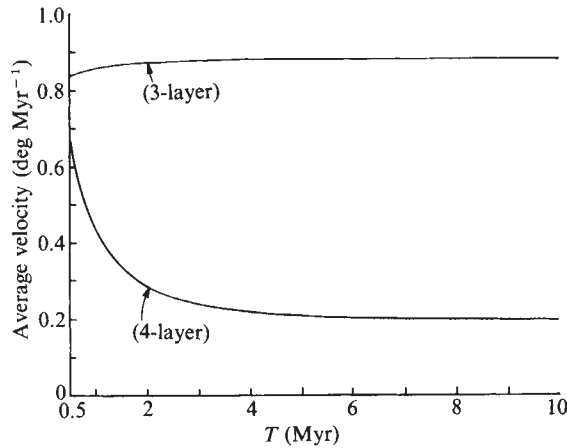


Fig. 2 A comparison of the averaged polar speed between the three- and four-layer models. A time interval of 10^5 yr is taken for the glacial cycle. The magnitude of the forcing in the Northern Hemisphere represents an ice volume which is equivalent to a 70-m rise of the global sea level; the meltwater contribution of the Southern Hemisphere is given by a 20-m rise of the sea level. A uniform mantle viscosity of 10^{22} P is used. The thickness of the elastic lithosphere is taken to be 100 km.

of inertia from forcing due to a surface point load which is maintained. The results between the two models are quite striking, as the transient responses, due to the M1 mode, linger on over a million years in the case of the four-layer model. On the other hand, for the three-layer model isostatic readjustment is completed within $0(10^4)$ yr. As the elastic lithosphere remains isostatically uncompensated in the final state, this gives rise to a finite asymptotic contribution to the perturbed inertia tensor.

This extremely long relaxation time should be also important in questions regarding the nature of the climatic response to perturbations in the Earth's orbital parameters^{11,12,17,18}. This is due to the fact that in the present climate models the process of viscous relaxation of the mantle in response to cryospheric forcing is described by simple half-space models¹⁹, which only produce a single relaxation time between 10^3 and 10^4 yr for mantle viscosities $0(10^{22})$ P. Hence, if one of the six quasistatic normal modes associated with ice dynamics is slower than those on which the orbital parameters vary $0(10^4-10^5)$ yr, this would imply that the nonequilibrium responses²⁰ of the climate system may be more important than previous conjectures.

The linearized solution of the Liouville equation^{14,21}, which describes the polar motion excited by surface loading for the four-layer model, can be cast in the Laplace-transformed domain as

$$\left[\frac{is}{\sigma_r} + 1 - \frac{1}{k_t} \left(K_0 + \sum_{i=1}^6 \frac{K_i}{s - s_i} \right) \right] \mathbf{m}(s) = \Phi(s) \quad (1)$$

where σ_r is the Chandler wobble frequency for the rigid Earth, k_t and K_0 respectively are the fluid and elastic limit of the tidal Love numbers² of spherical harmonic $l=2$, s_i is the inverse relaxation time of the i th mode of $l=2$, s is the Laplace transform variable; $\mathbf{m} = (m_1, im_2)^T$ are the direction cosines of the rotation axis relative to references axes through the Conventional International Origin (CIO)¹. Note that the present formulation takes into account, for the first time, both the transient responses from rotational deformation, uniformly valid with time, given in the left-hand side of equation (1) and from surface loading $\Phi(s)$. There are six modes of viscous gravitational relaxation in the four-layer model^{14,21} in which the ratio between the fastest and slowest relaxation times is several thousand. The fluid Love number k_t can be defined as

$$k_t = K_0 - \sum_{i=1}^6 \frac{K_i}{s_i} \quad (2)$$

The forcing function from the glacial loading is given spectrally by

$$\Phi(s) = \left(1 + I_0 + \sum_{i=1}^6 \frac{I_i}{s - s_i} \right) \mathbf{f}(s) \quad (3)$$

Here $\mathbf{f}(s)$ represents the time-dependent forcing from the second-degree harmonic component to the surface forcing¹. The contribution to the changes of the inertia tensor of the planet from elastic deformation due to this load is given by I_0 and I_i denotes the transient readjustment of the inertia tensor from relaxation of the j th mode^{1,14}. Substituting equation (2) into equation (1) we can reduce the expression to

$$s \left[1 + i\sigma_r \sum_{i=1}^6 \frac{K_i}{s_i(s - s_i)} \right] \mathbf{m}(s) = -i\sigma_r \left(1 + I_0 + \sum_{i=1}^6 \frac{I_i}{s - s_i} \right) \mathbf{f}(s) \quad (4)$$

The solution of equation (4) from partial fraction decomposition is given by

$$\mathbf{m}(s) = \left(\frac{A_1}{s} + \sum_{i=1}^6 \frac{A_{i+1}}{s - a_i} \right) \left(1 + I_0 + \sum_{i=1}^6 \frac{I_i}{s - s_i} \right) \mathbf{f}(s) \quad (5)$$

where $\{A_i\}$ are complex numbers²¹ obtained from solving a system of seven equations and $\{a_i\}$, representing the complex eigenvalues²¹ belonging to the spectrum describing rotational readjustment of the spin axis of a viscoelastic planet, are complex roots to a sixth-order polynomial in s . The set of complex eigenvalues has an essential role in the phenomenon of Chandler wobble excitation for a viscoelastic planet²². A saw-tooth waveform can be used to describe the glaciation-deglaciation cycle, which in the late Quaternary²³ is characterized by a very slow build-up of the major ice sheets with a time scale of 9×10^4 yr followed by a rapid disintegration over 10^4 yr. The time-dependence of this ramp-shaped function can be readily transformed to the Laplace transform domain¹ and be used in equation (5). The instantaneous speed of polar wander $d\mathbf{m}(t)/dt$ for a given cycle is obtained by performing the inverse Laplace transform of the velocity $s\mathbf{m}(s)$, which results in an expression involving several complicated series^{14,21}.

To study the impact of the M1 mode in the average polar speed $\bar{V}(T)$ the average velocity as a function of time T must be calculated by including the transient effects from long-term viscoelastic relaxation. At the onset of glaciation cycles the Earth is assumed to be in a state of isostatic compensation in so far as short-term ($t \leq 0(10^7)$ yr) perturbations are concerned. This is done by summing up over all of the glacial cycles involved. The average net motion is calculated by the expression

$$\bar{V}(T) = \frac{\int_0^T \frac{d\mathbf{m}(t)}{dt} dt}{\int_0^T dt} \quad (6)$$

where T represents the time since the onset of the ice-age cycles.

The average velocities for a three- and four-layer models are shown in Fig. 2 to show explicitly the effects of the phase transition on net polar wander. One observes that in the case of the three-layer model a steady-state speed is nearly established within a few cycles. However, for the model with the phase transition there is an initial transient of $0(1^\circ \text{ Myr}^{-1})$, which is followed by a subdued decay over a few million years to an asymptotic value of $0(0.1^\circ \text{ Myr}^{-1})$. This amounts to a reduction by about a factor of 4 from the results obtained for a model without a density discontinuity². This transient behaviour is ascribed to the presence of a 'slow' variable of the cryospheric-mantle system, namely, the M1 relaxation mode. Therefore, the average speed of polar wander today could conceivably be still at the initial transient stage, depending on the particular onset time²⁴ of large-scale continental glaciation in the Northern Hemisphere.

As there exists both empirical²⁵ and observational^{14,15} evidence to suggest that there is a weak viscosity stratification across the 670 km discontinuity, the effects of a slightly stiffer lower mantle on polar wander are examined in Fig. 3. We observe that when the viscosity in the lower mantle is increased

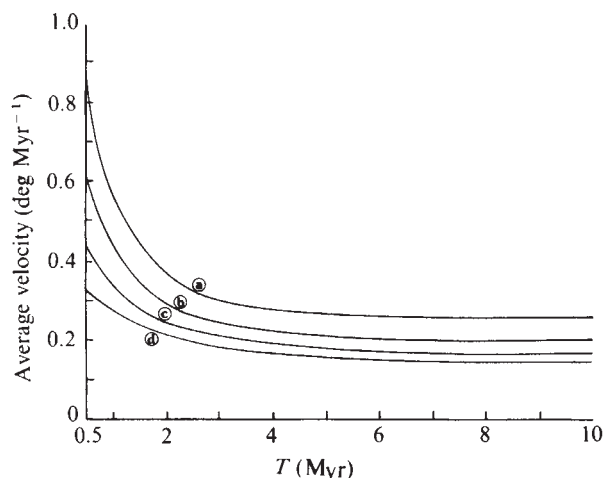


Fig. 3 Averaged velocity of polar wandering as a function of time for different lower mantle viscosities. The upper mantle viscosity (η_2) is fixed at 10^{22} P. The forcing parameter of the Northern Hemisphere is set to 90 m; otherwise, the parameters are the same as in Fig. 2. a, $\eta_1 = 10^{22}$ P; b, $\eta_1 = 2 \times 10^{22}$ P; c, $\eta_1 = 3 \times 10^{22}$ P; d, $\eta_1 = 4 \times 10^{22}$ P.

by a factor of 4, the net polar drift only decreases by about a factor of 2. This behaviour can be explained by the fact that more of the transient flow takes place preferentially in the upper mantle due to a stiffer lower mantle, thus yielding a compensatory contribution to the changes of the inertia tensor.

For this set of plausible mantle viscosity values²⁶, we find that cyclic forcing of the late Cenozoic ice ages can force a net drift of the rotation pole relative to the surface geography, at a rate of $0(0.1^\circ \text{ Myr}^{-1})$. This amount is a factor of about 4 times smaller than those derived from the three-layer models^{1,2}. We note that the instantaneous rates of polar wander as inferred from astronomical data^{27,28} are much higher than the current estimates from palaeomagnetic data²⁹ with TPW rates of between 0.2 and $0.3^\circ \text{ Myr}^{-1}$. These are, in fact, in close agreement with the steady-state values for the mean drift calculated here.

Our results for a more realistic Earth model reinforces the notion advanced by Sabadini *et al.*² that net polar displacement could be produced by glacial cycles. The main finding to which we wish to draw attention here is that TPW is influenced by the 'slow' physics of the system, brought about by a slowly decaying mode, which is ascribed to mantle phase transitions. Hitherto, this fact has not been considered in climate studies of ice ages. Geological evidence indicates the extent of glaciation from earlier ice ages in the Phanerozoic were of a comparable magnitude with those of the Pleistocene, whose forcing parameters have been used in this study. From the mean polar speed calculated above, this would imply that a significant amount of net polar drift $0(10^\circ)$, which would be sufficient to disrupt the geographical conditions conducive for maintaining glacial cycles, will eventually be attained in about 30–50 Myr. This length of time is comparable with the duration of earlier glacial epochs, such as those which occurred in the Permian and Silurian ages³⁰. We suggest that the characteristic time scale of major ice age epochs $0(10^7 \text{ yr})$ may be controlled by the slowest relaxing mode of the mantle, due to the presence of phase transitions.

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Parallel versus serial processing in rapid pattern discrimination

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When stimuli are available for just a brief period (~ 100 ms) only restricted spatial information can be processed by the visual system. If the stimuli are presented very briefly, eye movements are not possible. The time during which the after-image of the stimulus is available for inspection is terminated by presentation of a masking pattern. We show here that in these conditions a small pattern is easily detected against a background made up of many others, only if this target pattern differs from the background patterns in certain local features. In this case the detectability of the target is almost independent of the number of background elements, suggesting that a parallel process is operating. Detection of patterns not differing from their backgrounds in such features requires focal attention which is a serial process. The aperture of this attention is scaled to minimize the number of shifts of attention required.

Many factors determine the ease with which a visual pattern can be discriminated from those surrounding it. Julesz¹ has described a theory of preattentive texture discrimination based on a few conspicuous local features, which he called 'textons'. A similar theory has been proposed by Triesman and Gelade². An example of such preattentive texture discrimination is shown in Fig. 1A, in which (when viewed from up to about seven times picture height) the regions made of Xs and Ls in the upper half perceptually segregate while the regions made of Ls and Ts in the lower half do not. To determine the spatial configuration of these lower regions, element by element, scrutiny is required. We call this concentration on small areas 'focal attention'. Differences in the density of textons within a region give rise to preattentive texture discrimination while small differences in their positions do not. For example, the two non-overlapping perpendicular line segment textons have the same density in the regions made up of Ts and Ls and thus do not yield preattentive discrimination, although inspection by focal attention easily distinguishes between these regions.

We have investigated the relationship between preattentive texture discrimination and rapid pattern discrimination using a paradigm similar to that of Julesz and Burt³. In these experiments the stimuli consisted of a hexagonal array of high contrast white figures on a dark background (see Fig. 1B). The diameter of the hexagonal arrays is 15° arc; the line segments making

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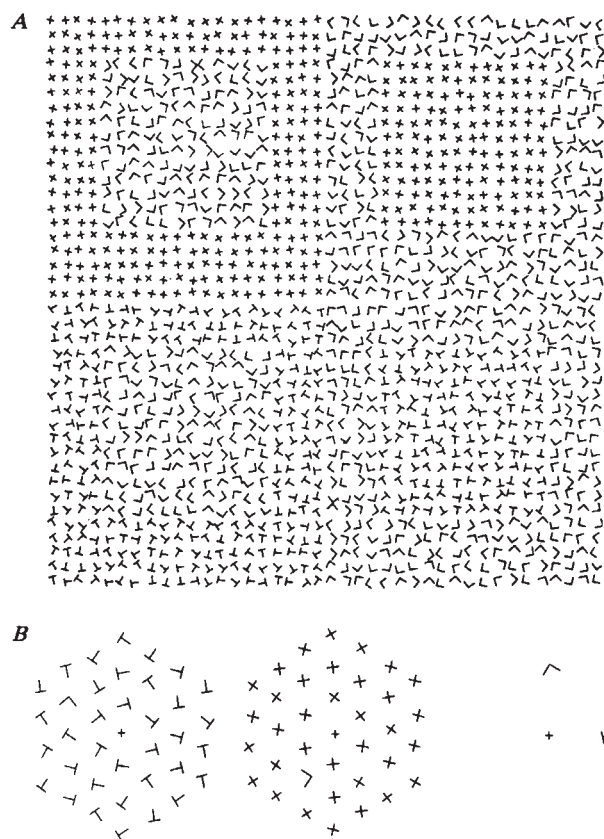


Fig. 1 *A*, Demonstration of preattentive versus attentive texture discrimination. The upper half of the figure contains distinct regions of clearly defined shape. The lower half contains embedded regions of identical size and shape which do not perceptually segregate. *B*, Stimuli of the type used in the experiments of Fig. 2*A* and *B*. Left, lower curve in Fig. 2*A*; middle, upper curve in Fig. 2*A*; right, first point of lower curve in Fig. 2*B*. (Actual stimuli were white figures on a dark CRT face.)

up the individual elements extend for 50 min arc. The task of the observer was simply to determine whether the elements were all the same, or if one, the 'target', was different. The target was present in half the trials. The position of the target element, if present, was varied randomly from trial to trial within the outer two shells of the hexagonal array. Thus, the observers did not know where it would appear next. The observer fixated the small central cross which was always present. The time available for inspection was limited by applying, after the test stimulus, an erasing flash consisting of elements which were the union of those to be discriminated. This procedure allows study of discrimination using inspection times shorter than the persistence of the after-image.

Figure 2*A* compares discrimination behaviour for the two elements which in Fig. 1*A* yield strong texture segregation, with that for two elements which do not. The test and masking patterns were each presented for 40 ms with a blank interval of variable duration between them. During this interval, the after-image of the test pattern was highly visible but could not, of course, be inspected using eye movements. The use of this brief flash presentation gives many of the benefits of retinal stabilization without sophisticated equipment. The data are plotted as a function of the interval between the test and mask onsets ('stimulus onset asynchrony' or SOA). The results in the two cases are clearly different. In the case of one L among 35 Xs (shown at the centre of Fig. 1*B*) detection is almost perfect by SOA of 160 ms, while for one L among 35 Ts (as in the example on the left of Fig. 1*B*) the asymptote is not reached until about 300 ms SOA and performance never exceeds about 62% correct.

Perceptually, the L among the Xs stands out, even when the inspection interval is very short, while the L among the Ts must

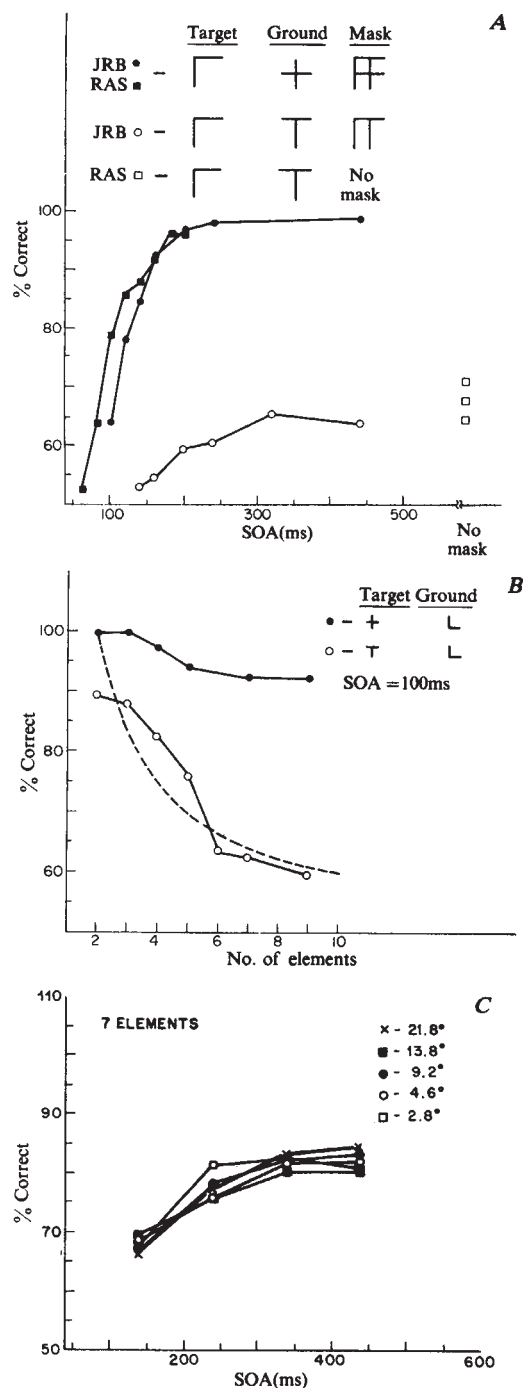


Fig. 2 *A*, Comparison of probability of correct detection of a target element in the presence of 35 background elements. Solid symbols represent the 'L' and 'X', which form strongly segregating textures, while the open symbols represent 'L' and 'T' elements, which do not (see Fig. 1*A*). Data for two experienced observers (J.R.B. and R.A.S.) are shown. The three points at the far right are three independent measurements of performance, without a mask, for R.A.S. *B*, Probability of correct detection of a single target element presented among a variable number of background elements. For description of dashed curve see text. *C*, Demonstration of scaling invariance. Seven elements were presented. Diameter of the stimulus ranged from 2.8 to 21.8 deg. arc.

be sought out. This introspective observation is supported by results for detection of the L when only one background T is presented, as shown on the right in Fig. 1*B*. In this case performance is similar to that for one L among 35 Xs. This suggests that the L among Xs somehow marks its own location, as if it were presented alone on a blank field. We believe that the texton difference between the X and L marks the location of the disparate element, removing the need to find it by serial

search. Based on the asymptotic performance level of 62% correct in the L among Ts case, we can estimate (as described below) the number of elements the observer was able to inspect to be about seven. (If we assume that the observer searches at random, but never inspects the same element twice, the probability of seeing a single target element among N total elements is n/N , where n is the number of elements inspected. At 62% correct discrimination, the probability that the target is seen is 25%, thus $n \approx 7$.) As the asymptote is reached by about 350 ms SOA (roughly the persistence time of the after-image⁴) we can then infer that it requires on average 50 ms to scrutinize a single element and move to another.

Further evidence for this parallel versus serial distinction is given by the data of Fig. 2B. Here the total number of elements presented was varied, and as described above, the task was to determine whether one of them was different. The inspection time was fixed at 100 ms to limit the amount of searching by focal attention. Once again, there was a very different pattern of results in the two cases. When the target was an X, the drop in discrimination performance when the number of background Ls was increased was slight. When the target was a T, however, performance fell off rapidly. The dashed curve approximating the latter set of points is based on the assumption that only two of the elements can be scrutinized in the 100-ms inspection interval, and that these elements are chosen randomly. (Based on the assumption that a given element is not inspected more than once, one derives a hypergeometric distribution giving the probability of seeing the target. In accordance with this distribution, the curve shown falls to chance performance as $2/n$, where n is the number of elements. This assumption also implies that the variance of the measured performance should be in accordance with the hypergeometric distribution; however, to test this more data would be required.) The rough correspondence between this curve and the data is consistent with a serial interpretation. The decline in detectability of the target with an increase in the number of background elements is also consistent with a parallel model if we assume that the signal-to-noise ratio falls as the number of background elements grows. In addition, it would have to be assumed that in the X versus L case no such noise increase occurs. Even with these assumptions it is not clear how this model would explain the difference in curve shapes seen in Fig. 2A.

As noted above, the serial search cannot involve eye movements, as the test field is presented for only 40 ms. It is rather the process by which the observer serially shifts a region of focal attention from one element to another, in order to identify it. As demonstrated by Posner⁵ among others, attention cannot simultaneously be devoted to multiple loci. The difference between the 'same textons' and 'different textons' cases is that in the latter, this search is immediately guided to the disparate element if it is present.

The discrimination behaviour described is independent of whether the stimulus falls entirely within the fovea or extends into the near periphery. This was tested over a range of a factor of 8 in size using stimuli consisting of seven elements arranged randomly in a ring centred at the fixation mark. These elements were either all Ts, all Ls, 1 L and 6 Ts, or 1 T and 6 Ls, chosen at random with equal probability. As before, the observer was required to report whether all elements were the same or not. The smallest of these stimuli subtended only 2.8° of visual angle, while the largest was nearly 22° in diameter. Figure 2C shows that this uniform contraction or dilation of the stimulus had little effect on performance. Thus, within the limits imposed by spatial resolution, the stimulus can be viewed from a wide range of distances with similar results. One type of scaling invariance has been well known for over a century (according to the 'Aubert-Foerster law') to apply in experiments involving spatial resolution⁶ (an observer fixating the same point from different distances will generally report that an eccentrically placed letter which is resolvable from one distance will remain so when the viewing distance is changed). This suggests that except for the change of spatial scale, the fovea and near periphery function

similarly in the extraction of visual information. The invariance reported here further suggests that the fovea is not especially privileged with respect to the allocation of attention. It was demonstrated by Posner *et al.*⁷ that extrafoveal attention produces as much decrease in the detectability of foveal targets as does foveal attention on the detectability of extrafoveal targets. This idea is extended by the findings reported here, in that the domain of attention can be a small portion of the fovea, only a few minutes of arc in diameter.

These results clearly have implications for interpretation of the large body of data on visual search and detection. Many psychologists have proposed higher level cognitive theories of pattern (usually letter) detection and discrimination which might account for the results presented here. We have not discussed these alternative interpretations because we feel that a much simpler model such as the one described here is sufficient. Of course, there may be much discrimination behaviour that requires explanation in terms of high level cognitive constructs. We suggest, however, that due to the highly variable texton content of letters, the interpretation of many experiments in which the stimuli are arrays of letters or words will be complicated.

The present results suggest that two different processes are involved in perception of briefly inspected patterns. One is a parallel process operating over large areas while the other is serial, limited to a restricted region depending on the stimulus size. Discrimination of certain feature differences can be accomplished rapidly by the former process while other differences require more lengthy serial scrutiny. Interestingly, the same feature differences that yield preattentive texture discrimination are also those detectable by the rapid parallel process. While these findings are consistent with the texton theory mentioned at the outset, the inference of two distinct processes at work does not depend on any specific theory of texture perception.

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Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat

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Eyespot disease, caused by the fungus *Pseudocercospora herpotrichoides*, is responsible for considerable lodging and reductions of yield in extensive areas of wheat cultivation in North and South America, Europe, New Zealand, Australia and Africa¹. The level of resistance of wheat cultivars is too low, even among the less susceptible ones (that is, Cappelle Desprez and Cerco) and no genes for resistance have to date been characterized in any species. Sprague² found a high level of resistance to this disease in the wild grass *Aegilops ventricosa* and several workers have attempted its transfer to cultivated wheat with only partial success³⁻⁵. We report here a major dominant gene for resistance, which has been transferred from tetraploid *Ae. ventricosa* (genomes $D^*D^*M^*M^*$) to hexaploid wheat, *Triticum aestivum* (AABBDD), using tetraploid wheat, *Triticum turgidum* (AABB), as a 'bridge' species.

The breeding scheme is represented in Fig. 1. A hybrid was obtained between the donor and the bridge species, which was male sterile, probably due to the lack of homology between the genome complements of its progenitors. The meiosis of this hybrid was irregular and the egg cells generated presumably had a wide range of chromosome combinations. Certain of these egg cells yielded seeds when the hybrid was pollinated with pollen from hexaploid wheat. Plants from these seeds were fertile and, after repeated selfing, stable lines with 42 chromosomes (H-93-1 to H-93-70) were derived from them. These lines were screened for biochemical markers encoded by genes located in the D^v and M^v genomes of *Ae. ventricosa*⁶⁻⁸. Due to the partial homology of the D^v genome of the donor and the D genome of the recipient, markers associated with the D^v genome appeared at high frequencies (30–60%). Genes from the M^v genome, which is not homologous to any of the hexaploid wheat genomes (A , B or D), generally appeared at lower frequencies (<4%), although one such gene did appear at a higher frequency (31%)⁷. A study of meiosis in hybrids between H-93 lines and the hexaploid wheat, together with the biochemical data, indicated that genes from the donor had been incorporated into the H-93 lines both by chromosomal substitution and by recombination^{6,7}. Attempts at direct hybridization between *Ae. ventricosa* and hexaploid wheat had been unsuccessful at the time the initial crosses described here were performed. The only such hybrid subsequently obtained was male sterile⁹. This hybrid would probably have been a less adequate intermediate in the gene transfer because it carried all the hexaploid wheat genomes (A , B and D) and, when rescued with ABD pollen, would have had a greater tendency to eliminate the alien genetic material while selecting for the euploid chromosome number ($2n = 42$).

A high proportion of the H-93 lines were found to be highly resistant to the disease (Fig. 2 and ref. 8) and line H-93-70 was selected for further study because of its good performance in tests for resistance both at the seedling and at the adult stages (Table 1). Reciprocal crosses (F_1 and F_2 generations) and backcrosses were obtained from line H-93-70 and the recipient *T. aestivum* cultivar Almantense H-10-15 (hereafter designated H-10-15) and tested for resistance. Two types of observation were made: a quantitative one, the number of leaf sheaths affected per plant, and a qualitative one, the type of mycelium.

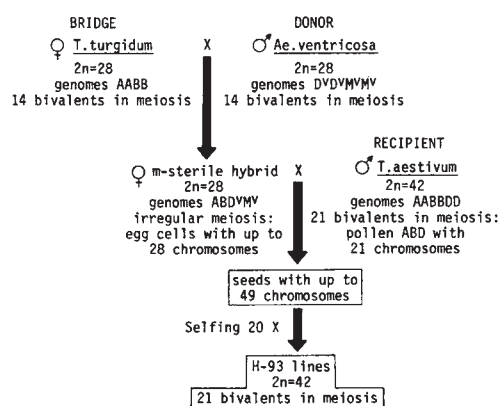


Fig. 1 *Triticum turgidum* H-1-1, *Aegilops ventricosa* AP-1 and *T. aestivum* cv. Almantense H-10-15 were crossed according to this breeding scheme. Seeds resulting from these crosses were selfed repeatedly until a wide range of stable morphological types had been obtained. After stability had been achieved, somatic chromosome numbers were counted in the root tips of germinating seeds stained by the Feulgen procedure. Most of the lines had the euploid number of chromosomes ($2n = 42$) at that stage. The distribution among these lines of biochemical markers corresponding to the D^v and M^v genomes of the donor species was then investigated^{6,7}. Subsequently, susceptibility to eyespot disease was tested in the same lines⁸.

The pathogen, which is relatively nonspecific, produces two types of mycelium, depending on the host¹⁰⁻¹²: on susceptible plants, the mycelium is abundant between the leaf sheaths, and is coloured black (m type); on resistant plants with *Ae. ventricosa* cytoplasm the mycelium is spotted and dark-brown (v -type). Leaf sheaths of resistant plants are invaded more slowly than those of susceptible ones and often they are not invaded beyond the first leaf, so that no stroma can be observed. Sometimes the distinction between the two types of mycelia is not clear-cut.

Table 2 summarizes the results of these tests. In our experimental conditions, close to 90% of the H-93-70 plants tested had two or less leaf sheaths infected, whereas a similar proportion of plants from H-10-15 had from three to five leaf sheaths infected. Both the pattern of segregation and the average number of leaf sheaths penetrated in F_1 , F_2 and backcross

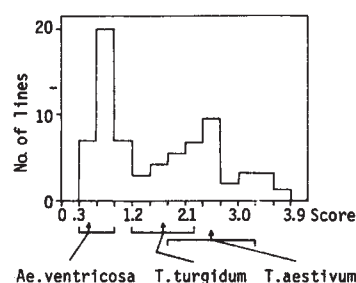


Fig. 2 Susceptibility to *Pseudocercospora herpotrichoides* (score: average number of leaf sheaths infected per plant) of H-93 lines (H-93-1 to H-93-70) and of the parental material from which they were derived. The arrows indicate the average value for each of the parental species and the brackets the range of values that do not differ significantly from those averages ($P < 0.5\%$). Scores for 33 of the lines did not differ significantly from that of *Aegilops ventricosa* AP-1 and were significantly lower than that of *Triticum turgidum* H-1-1, the most resistant of the two wheat progenitors. Only four lines were more susceptible than *T. aestivum* cv. Almantense H-10-15.

Table 1 Susceptibility to eyespot of line H-93-70 and its progenitors

Stocks	Seedling stage*†	Adult stage††
H-93-70	0.367 a	0.221 a
<i>Ae. ventricosa</i> no. 11	0.366 a	0.356 b
<i>Ae. ventricosa</i> AP-1	0.404 a	0.373 b
VPM-1112-R4	0.808 a	0.399 b
<i>T. turgidum</i> H-1-1	1.713 b	0.453 b
<i>T. aestivum</i> cv. Cappelle Desprez	2.220 b	0.503 bc
<i>T. aestivum</i> cv. Almantense H-10-15	2.540 c	0.585 c

In addition to line H-93-70 and its progenitors, the following stocks were tested: Accession no. 11 (Rennes) of *Ae. ventricosa*, line VPM-1112-R4, which is a derivative of line VPM1, and cultivar Cappelle Desprez, which is the less susceptible hexaploid wheat. Line VPM1 was obtained by crossing a synthetic allopolyploid ($2 \times [\text{♀ } Ae. ventricosa \times \text{♂ } T. persicum]$; genomes $AABBD^vD^vM^vM^v$) with a hexaploid wheat³ and has a level of resistance which is intermediate between those of *Ae. ventricosa* and wheat.

* The average numbers of leaf sheaths attacked per plant are given. Three replicates of 50 seeds each were sown in a plastic tunnel and inoculated with mycelium which had been obtained by *in vitro* culture and ground into a powder⁸.

† Duncan's multiple range test was used; numbers followed by different letters in the same column differ significantly ($P < 0.5\%$); variation coefficients were 22% and 24.8% in the tests at the seedling and the adult stages, respectively.

‡ The proportions of stems with more than 50% of section attacked are given. Three replicates of 50 seeds each were sown in the field and inoculated by spraying a suspension of the fungus previously cultivated on oat grains⁸.

Table 2 Inheritance of resistance to eyespot disease in crosses of line H-93-70 with *T. aestivum* cv. Almatense H-10-15

Sample	Cytoplasmic background	No. of plants No. of leaves affected*			Mean of no. of leaves affected		Mycelium type‡ (% plants)		
		Tested	0-2	3-5	Observed	Expected†	(-)	(v)	(m)
Expt 1									
H-93-70	<i>T. turgidum</i>	30	27	3	1.60		43.3	56.7	0.0
H-10-15	<i>T. aestivum</i>	30	3	27	3.33		0.0	0.0	100.0
F ₁ (♀H-93-70 × ♂H-10-15)	<i>T. turgidum</i>	30	27	3	1.90		13.3	83.3	0.0
F ₁ (♀H-10-15 × ♂H-93-70)	<i>T. aestivum</i>	30	23	7	2.07		23.3	53.4	23.3
F ₂ (♀H-93-70 × ♂H-10-15)	<i>T. turgidum</i>	178	128	50	2.05	2.18	25.8	65.2	9.0
Expt 2									
F ₂ (♀H-10-15 × ♂H-93-70)	<i>T. aestivum</i>	116	86	30	2.22	2.26	2.6	69.0	28.4
(♀H-10-15 × ♂H-93-70) × ♂H-93-70	<i>T. aestivum</i>	89	85	4	1.69	1.83	5.6	88.8	5.6
(♀H-10-15 × ♂H-93-70) × ♂H-10-15	<i>T. aestivum</i>	88	40	48	2.68	2.70	1.1	42.0	56.8
Expt 3									
H-93-70	<i>T. turgidum</i>	95	82	13	1.75		45.3	53.6	2.1
H-10-15	<i>T. aestivum</i>	99	15	84	3.22		3.0	6.1	91.0
F ₁ (♀H-93-70 × ♂H-10-15)	<i>T. turgidum</i>	100	73	27	2.04		13.0	86.0	1.0
(♀H-93-70 × ♂H-10-15) × ♂H-93-70	<i>T. turgidum</i>	98	80	18	1.91	1.90	33.7	65.3	1.0
(♀H-93-70 × ♂H-10-15) × ♂H-10-15	<i>T. turgidum</i>	97	49	48	2.57	2.63	9.2	64.0	26.8

F₁, F₂ and backcross generations were obtained with two different cytoplasmic genetic backgrounds: that of line H-93-70, which had been originally contributed by *T. turgidum* H-1-1 (see Fig. 1), and that of the *T. aestivum* cv. Almatense H-10-15. Plants were tested at the seedling stage.

* Plants were classified into resistant (two or less leaf sheaths infected) and susceptible (three to five leaf sheaths infected). The observed segregation in each of the F₂ generations did not differ significantly ($\chi^2 \ll \chi^2_{df=1, P=0.05} = 3.84$) from the segregation 3 resistant/1 susceptible expected for a dominant gene. The observed segregations in each of the backcrosses to the susceptible parent (H-10-15) did not differ by the same criterion from the segregation 1 resistant/1 susceptible expected for a dominant gene.

† Expected means for F₂ generations and backcrosses were calculated from the observed means of the progenitors and the appropriate F₁ generation from the same experiment, except for experiment 2, in which the data from experiment 1 were used.

‡ No stroma observed (- type); spotted and dark brown mycelium (v type); abundant black mycelium (m type).

generations indicated that resistance was inherited as though determined by a single mendelian factor (*Pch1*), which showed almost complete dominance.

In F₁, F₂ and backcross generations, the proportion of plants with black mycelia (m type) was greater when the cytoplasmic genetic determinants (mitochondrial and chloroplast genomes), which are maternally inherited, were contributed by *T. aestivum* cv. Almatense H-10-15 than when the source was H-93-70, whose mitochondrial and chloroplast genomes were donated by *T. turgidum* H-1-1, the bridge species used as a female in the transfer scheme (Table 2). The proportions of mycelium types seem to be more variable in response to environmental conditions, or in relation to plant vigour, when the *Pch1* gene is expressed in the H-10-15 cytoplasmic genetic background. This effect is less conspicuous when resistance is assessed by the number of leaves penetrated. A similar effect on resistance to that reported here for the *T. turgidum* H-1-1 cytoplasmic genetic background has previously been described for the *Ae. ventricosa* background¹⁰⁻¹².

To investigate whether the *Pch1* gene in H-93-70 was integrated in a chromosome capable of pairing and recombination with wheat chromosomes, meiosis of the hybrid ♀ H-93-70 × ♂ H-10-15 was studied and was found to be quite regular: 20.70 ± 0.02 bivalents at metaphase I in the hybrid, versus 20.82 ± 0.05 bivalents in H-93-70 and 20.98 ± 0.02 bivalents in H-10-15 (at least 5 plants and 50 cells were analysed in each case; differences between the hybrid and the progenitors were not significant). These observations exclude the presence in H-93-70 of whole chromosomes or large chromosomal segments non-homologous to wheat chromosomes and indicate that the *Pch1* gene can be introduced by recombination into other wheats. The present data cannot distinguish whether the *Pch1* gene has been transferred from the *M*^v or the *D*^v genomes of *Ae. ventricosa* because, although resistance was transferred with the high frequency expected of a *D*^v gene, recombination between *D*^v and *M*^v chromosomes before the transfer has been suspected in *Ae. ventricosa* AP-1^{6,7,12}.

The chromosomal locations of resistance factors in cultivar Roazon, whose intermediate level of resistance has been acquired from line VPM1 (see Table 1 legend), have been assigned to chromosomes 7A, 2B, 5D and 7D, the main effect being associated with the last chromosome. It will be of interest

to investigate whether gene *Pch1* in H-93-70 is also located in chromosome 7D.

Law *et al.*¹³ demonstrated that more than one chromosome (gene) was involved in the differences in susceptibility between Cappelle, the most resistant wheat cultivar, and other wheat cultivars. They detected both positive and negative effects that were dependent on the genetic background. The higher level of resistance attained in the present case, as compared with previous attempts³⁻⁵, can be explained in terms of the monogenic nature of the resistance transferred and of the nature of the genetic background, nuclear as well as cytoplasmic, into which the transfer was made: *T. aestivum* cv. Almatense H-10-15 is almost as resistant as Cappelle, and *T. turgidum* H-1-1, the cytoplasm donor, is even more resistant.

Apart from contributing to our understanding of the genetic control of resistance to eyespot disease, the gene reported here should be immediately useful in converting susceptible wheat cultivars to this major disease resistant ones.

This paper is dedicated *In Memoriam* to M. Alonso Peña who carried out the initial crosses. We acknowledge the technical assistance of H. Joualt, C. Pérez-Pellon and C. Rojas.

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Co-expression of vimentin and cytokeratins in parietal endoderm cells of early mouse embryo

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Of the five classes of intermediate filaments found in vertebrate tissues, the cytokeratins are considered unique to epithelial tissues, while vimentin is expressed by endothelial and mesenchymal cells¹. In neither case is the precise function of the filament system known. Epithelial cells in culture often express vimentin as well as cytokeratins¹⁻³, but co-expression *in vivo*, as reported for pleomorphic adenomas of the parotid gland^{4,5} and metastatic carcinoma cells in ascites or pleural fluid⁶, is still controversial. Here we report the co-expression of cytokeratins and vimentin *in situ*, in the parietal endoderm of the mouse embryo 8.5–13.5 days old. This population of individual, motile cells seems to be derived from a conventional epithelium by migration and differentiation. Our results support the idea that vimentin expression is specifically related to reduced cell-to-cell contact, and to the independent existence of a cell following detachment from an epithelial sheet.

Parietal endoderm (PE) cells first appear in the 4.5-day-old mouse blastocyst and are thought to arise from a layer of bipotential precursor cells known as the primitive endoderm, which overlies the embryonic ectoderm^{7,8}. The PE cells migrate out onto the inner surface of the trophectoderm and form a pure population of cells synthesizing a thick basement membrane known as Reichert's membrane (for review see ref. 9). Those primitive endoderm cells, which remain in contact with the embryonic ectoderm, form a continuous, simple epithelium,

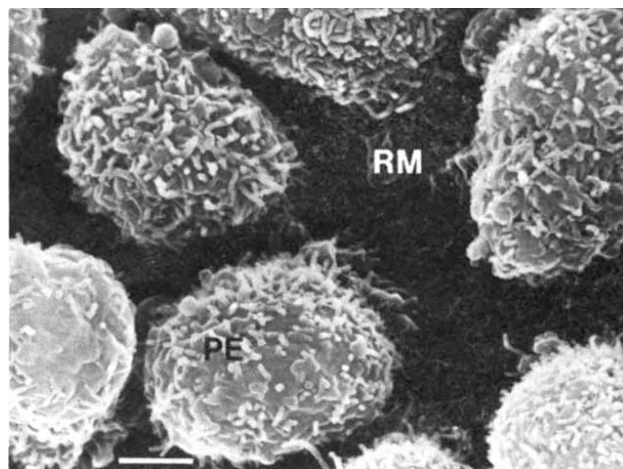


Fig. 1 Absence of extensive cell-to-cell contacts between parietal (distal) endoderm (PE) cells of a 13.5-day-old C3H/He mouse embryo, attached to Reichert's membrane (RM), viewed by scanning electron microscopy. Day of vaginal plug = day 1 of gestation. Tissue was fixed with 6% glutaraldehyde *in situ* immediately after dissection, critical point dried with CO₂ and sputter-coated with 15 nm palladium. Scale bar, 2 µm.

specialized for secretion and absorption, known as the visceral (proximal) endoderm^{7,10-12}. Both the primitive and visceral endoderm cells are joined by apical junctional complexes, but parietal endoderm cells have neither extensive nor specialized intercellular contacts^{7,10,11} (see Fig. 1). They have recently been shown by time-lapse cinematography to move independently of each other (D. Cockcroft, personal communication). Although conclusive evidence is not yet available, there is reason to believe^{8,12,13} that even after 4.5 days of development, PE cells continue to be generated from cells in the visceral endoderm layer, by detachment and migration from the epithelial sheet.

Work from several laboratories has shown that cytokeratin intermediate filaments first appear in the developing mouse embryo in the trophectoderm of the blastocyst, and subsequently in the extraembryonic endoderm and embryonic

Fig. 2 Cytokeratin (A,B) and vimentin (C,D) filaments seen in PE cells *in situ* by indirect immunofluorescence; the two filament systems show characteristically different patterns. The basket-like network of cytokeratin filaments extends throughout the cell, whereas the vimentin filaments are more condensed near the nucleus. Over 99% of all cells are stained in both cases. Identical results were obtained with 8.5-, 12.5- or 13.5-day-old embryos. Dissected Reichert's membranes with attached PE cells were spread out on plastic tissue culture dishes, fixed with 1:1 methanol/acetone (10 min at room temperature), air-dried and stained for indirect immunofluorescence immediately or stored at -70 °C until used. A, B, 13.5-day PE cells on Reichert's membrane stained with monoclonal antibody LE65 (ref. 18), using cell supernatant for 45 min at room temperature and Miles-Yeda rabbit anti-mouse fluorescein-conjugated second antibody (1:40 dilution, 30 min at room temperature). LE61 gives the same pattern. C, D, 8.5-day PE cells stained with rabbit anti-calf lens vimentin antiserum¹⁹ at 1:15 dilution for 45 min at room temperature. Second antibody was Cappel goat anti-rabbit, fluorescein-conjugated (1:40, 30 min at room temperature). The same pattern was seen with either of the two anti-hamster vimentin antisera^{20,21}. Scale bars, 10 µm.

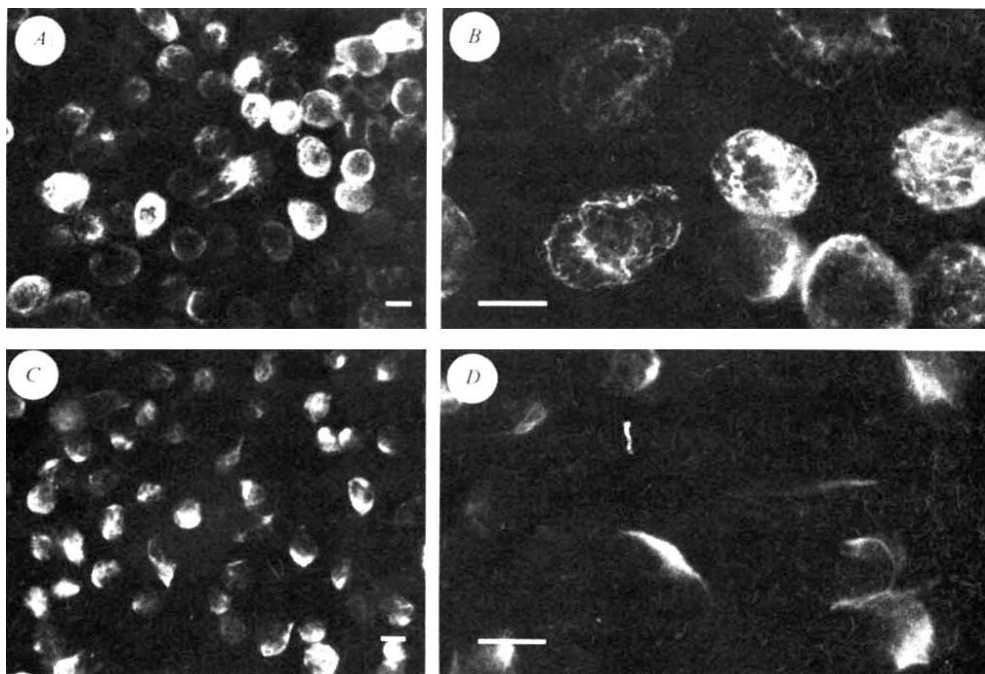
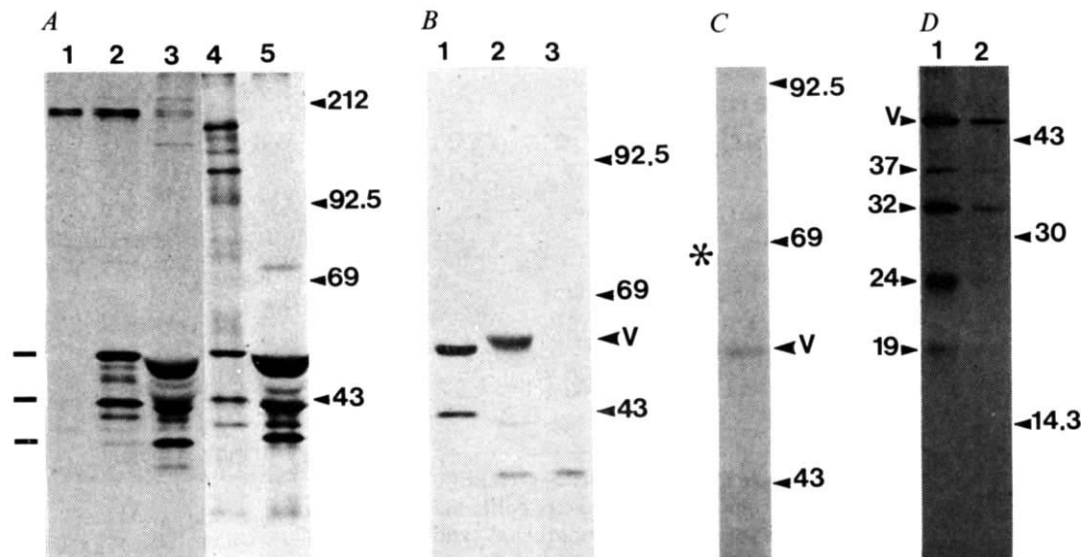


Fig. 3 **A**, Synthesis of cytokeratins by PE cells. Tracks 1–3 are ^{35}S -methionine-labelled polypeptides immunoprecipitated from the soluble fraction of PE cells. Track 1, normal rabbit serum was used; the two 200K polypeptides are non-specifically precipitated type IV procollagen. Track 2, monoclonal antibody LE65 (LE61 gave the same result); and track 3, rabbit anti-keratin antiserum. In tracks 3 and 5, where polyclonal antisera were used, the migration of the keratin polypeptides is distorted by the large amount of unlabelled immunoglobulin heavy chains (M_r 53K) in the immunoprecipitate. Such distortion was not seen when the monoclonals were used. Tracks 4 and 5 (from the same gel as 1–3) are immunoprecipitates from total *in vitro* translations of poly(A)⁺ RNA from PE cells, using monoclonal antibody LE65 (track 4) or rabbit anti-keratin antiserum (track 5). The identities of the high-molecular weight polypeptides in track 4 are not yet known. Bars on the left-hand side indicate the three major cytokeratin polypeptides immunoprecipitated by the various anti-keratin antibodies, with M_r (top to bottom) of 52–54K, 44–46K and 37–40K. The position of the ^{14}C -labelled molecular weight markers (Amersham), which were included in each gel, are as indicated ($M_r \times 10^{-3}$) from top to bottom; myosin, phosphorylase B, serum albumin and ovalbumin. **B**, Synthesis of vimentin by PE cells. Cells were labelled with ^{35}S -methionine for 1 h and polypeptides immunoprecipitated from the insoluble fraction as described below. Track 1, monoclonal antibody LE65; track 2, rabbit anti-vimentin antiserum¹⁹. In track 2, in addition to the major 56K vimentin (arrowhead labelled V) some smaller molecular weight species were immunoprecipitated; these were variable in amount and always correlated with amounts of the same bands brought down by normal rabbit serum (track 3). **C**, Identification of vimentin by immunoblotting. This shows an immunoblot of the anti-calf lens vimentin antiserum¹⁹ binding to a soluble extract of PE cells. Identical results were obtained with another²⁰ anti-vimentin antiserum. Antibody binding was visualized by autoradiography of ^{125}I -labelled Fab fragments of donkey anti-rabbit IgG (Amersham). Molecular weights were calculated from internal pre-stained molecular weight standards (BRL) which were transferred to the blot. Arrowhead labelled V indicates vimentin; the asterisk indicates weak cross-reactivity with a species of M_r 68K (see text), which was seen only on the blots and not in the immunoprecipitations. **D**, Partial peptide map of vimentin from 3T3 (track 1) and PE (track 2) cells. Arrowhead labelled V indicates uncleaved vimentin. The position of the ^{14}C -labelled molecular weight markers (Amersham) included in the gel are as indicated, top to bottom ($M_r \times 10^{-3}$) on the right-hand side: ovalbumin, carbonic anhydrase and lysozyme.



Methods: **A**, Groups of about 20 membranes were dissected from 13.5-day-old embryos, carefully cleaned of the few residual trophoblast cells, washed and incubated for 1 h in Dulbecco's modified Eagle's medium without methionine but containing 10% dialysed fetal bovine serum and $100 \mu\text{Ci ml}^{-1}$ ^{35}S -methionine ($1,000 \text{ Ci mmol}^{-1}$; Amersham). The cells were then washed in Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline (PBSA) and lysed in 0.5 ml of 0.05 M Tris-HCl, pH 8.0 with 0.15 M NaCl, 0.005 M EDTA and 1% Nonidet-P40 (NP40; lysis buffer), then centrifuged at $10,000g$ for 5 min, all at 4°C . The supernatant fraction was retained and the pelleted residue washed twice in lysis buffer before dissolving in 0.5 ml of sample buffer (0.065 M Tris, pH 6.8 with 2% SDS and 5% mercaptoethanol) at 100°C for 3 min. Aliquots of the supernatant and pellet fractions containing $\sim 3 \times 10^5$ c.p.m. TCA-precipitable radioactivity were diluted into 1 ml of 0.4 M NaCl, 0.005 M EDTA, 0.05 M Tris, pH 8.0 with 1% NP40, and polypeptides immunoprecipitated using 3–5 μl of specific antisera and excess protein A-agarose (Sigma) as described elsewhere³¹. Samples were analysed on SDS 10% polyacrylamide gels in reducing conditions followed by fluorography. Quantitation of newly synthesized cytokeratins in the supernatant and pellet fractions was carried out as described elsewhere³², using the rabbit antiserum against human callus keratin. Control experiments showed that cytokeratin polypeptides retained their antigenicity after heating to 100°C in SDS and mercaptoethanol. Isolation of poly(A)⁺ RNA from PE cells and its translation in the nuclease-treated rabbit reticulocyte lysate were carried out as described previously³³. **D**, Approximately 7×10^6 cells (10^5 cells per Reichert's membrane; 3T3 cells growing exponentially) of each kind were labelled for 2 h with $500 \mu\text{Ci ml}^{-1}$ ^{35}S -methionine. Cells were washed in PBSA and lysed in 0.8 ml of sample buffer at 100°C for 3 min, and DNA sheared by passage through a narrow gauge needle. Immunoprecipitates with anti-vimentin antiserum¹⁹ were run on a SDS 10% polyacrylamide gel which was then exposed to X-ray film without fixation; localized 56K bands were excised and incubated for 30 min at room temperature with 0.015 M *n*-chlorosuccinamide in urea as described elsewhere²⁵. After extensive washing of the gel slices, the cleaved polypeptides were electrophoresed out of the slices into a 20% SDS gel, and the peptides visualized by fluorography.

ectoderm^{10,14–16}. It has been reported that vimentin filaments do not appear until late on the eighth day of development, and then only in the primary mesoderm^{10,17}; these cells were found to be keratin-negative, which suggested sequential and exclusive expression of keratin and vimentin genes during the differentiation of mesoderm from the epithelial embryonic ectoderm.

To study the expression of intermediate filaments by PE cells, we dissected Reichert's membranes from 8.5–13.5-day-old mouse embryos cleanly away from the embryo and trophoblast. For immunofluorescence, membranes were pinned out and fixed (see Fig. 2 legend), allowing almost the whole population of PE cells to be viewed *en face*, rather than a few cells at a time as in sectioned embryos. For biosynthetic experiments the unfixed membranes were incubated for 1 h with ^{35}S -methionine, followed by immunoprecipitation of cell extracts (see Fig. 3 legend). Figure 2A, B shows that the mouse monoclonal anti-

cytokeratin antibodies LE61 and LE65 (ref. 18) reveal a network of filaments in virtually all PE cells. Both monoclonal antibodies and a polyclonal rabbit antiserum raised against human plantar callus keratin² immunoprecipitate newly synthesized polypeptides which fall within the size range of simple epithelial cytokeratins. The sizes of the three major polypeptides were calculated to be molecular weight (M_r) 52,000–54,000 (52–54K), 44–46K and 37–40K, depending on the gel (Fig. 3). No higher molecular weight cytokeratin polypeptides were synthesized by PE cells that were recognized by the various antibodies used here.

In the immunoprecipitation experiments described above, after 1 h of labelling $\sim 92\%$ (average of four experiments) of the newly synthesized cytokeratins of PE cells were recoverable from the $10,000g$ supernatant fraction (see Fig. 3 legend). No qualitative difference was seen between the cytokeratin polypeptides present in the supernatant versus the pellet fractions.

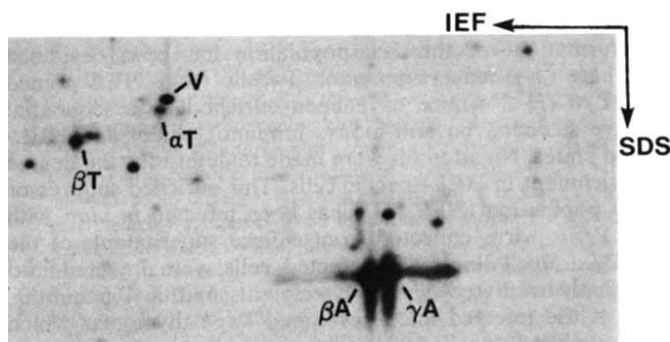


Fig. 4 Synthesis of vimentin by PE cells revealed by two-dimensional gel electrophoresis. Reichert's membranes from 10-day-old embryos were dissected and labelled overnight. A sample of the soluble fraction was analysed as described elsewhere²¹ by isoelectric (IEF) focusing on a pH 5–7 gradient followed by SDS-polyacrylamide (10%) slab gel electrophoresis. Part of the whole gel autoradiograph is shown. V, Polypeptide migrating in exactly the same relative position as vimentin from a variety of other cell types, including L6 rat myoblasts²² and B103 rat neural cells²¹. β A, γ A: β - and γ -actin, respectively; α T, β T: α - and β -tubulin. The acidic end of the gradient is to the left of the figure, the basic end to the right.

Evidence that these cytokeratins were being synthesized *de novo*, and that their presence in PE cells was not merely due to slow turnover of proteins made by cells at an earlier stage of development, came from the *in vitro* translation of parietal endoderm poly(A)⁺ RNA in the nuclease-treated reticulocyte lysate (see Fig. 3A, tracks 4 and 5). Immunoprecipitation of the total translation products with polyclonal or monoclonal antibodies recovered polypeptides which co-migrated with the ³⁵S-methionine-labelled cytokeratins synthesized by intact parietal endoderm cells.

When PE cells from 8.5–13.5-day-old embryos are stained *in situ*, without culturing, with any one of three independently raised vimentin antisera^{19–21}, a filamentous pattern is seen which is distinct from that of the cytokeratin filaments (compare Fig. 2D with B) and is characteristic of vimentin arrays in other cell types.

Several other lines of evidence confirm that PE cells are expressing vimentin. First, immunoprecipitation of both supernatant (not shown) and pellet (Fig. 3B, track 2) fractions of ³⁵S-methionine-labelled PE cells with vimentin antibodies reveals synthesis of a major polypeptide of M_r ~56K, corresponding in size to vimentin. Second, high-resolution two-dimensional gel electrophoresis of ³⁵S-methionine-labelled parietal endoderm cell extracts (Fig. 4) shows the presence of a polypeptide having coordinates which correspond exactly to those of vimentin, as seen in various other cell types^{22,23}.

Third, immunoblotting experiments²⁴ show anti-vimentin antibody binding to a single major species of M_r 56K (Fig. 3C). A minor reaction is also seen with a 68K polypeptide, as reported by others²⁵ for different anti-vimentin antisera applied to a tumour. It was suggested²⁵ that this 68K species might be a cytokeratin usually expressed in epidermis. In our case this explanation seems unlikely, as no labelled polypeptide of this molecular weight was immunoprecipitated from PE cells by the polyclonal anti-epidermal keratin serum (see Fig. 3A, track 3). No high molecular weight cytokeratins have been detected in such mouse embryos by others^{10,17}, and all three antisera are non-reactive with sections of epidermis (ref. 19 and our unpublished observations). We cannot rigorously exclude the possibility that the immunofluorescence staining of PE cells by the anti-vimentin antisera used for immunoblotting^{19,20} was due to cross-reaction with the 68K species seen here in the immunoblots, whatever its nature. However, the corollary of this, that the PE cells which are synthesizing vimentin are not organizing it into filaments, seems most unlikely. Moreover, the filament patterns revealed by anti-cytokeratin and anti-vimentin antibodies in PE cells are quite different (see Fig. 2).

Finally, evidence that the 56K polypeptide synthesized by PE cells and immunoprecipitated by anti-vimentin antisera has a similar chemical structure to the vimentin of mouse 3T3 cells is shown in Fig. 3D. Treatment of the ³⁵S-methionine-labelled 56K polypeptide from both PE and 3T3 cells with *n*-chlorosuccinamide²⁶ yields four polypeptides of M_r ~37K, 32K, 24K and 19K. This partial peptide map is consistent with cleavage of vimentin at both the unique tryptophan residue near the centre of the molecule and at the unique cysteine about 40 residues further towards the carboxy-terminal (ref. 27 and K. Weber, personal communication). It is also consistent with recent data suggesting that mammalian cells have only a single vimentin gene per haploid chromosome set²⁸.

It is clear from our observations that cells of the parietal (distal) endoderm synthesize two distinct intermediate filament systems, one of a type identifiable as vimentin, the other consisting of cytokeratin proteins. In other experiments, trophoblast giant cells were found to contain only cytokeratin filaments, and no vimentin, as reported by others^{15,16}. In the 13.5-day amnion, the ectoderm-derived epithelial layer expressed only cytokeratin while the mesoderm-derived cells expressed only vimentin (E.B.L. and B.L.M.H., unpublished observations).

Vimentin intermediate filament expression is seen in most, but not all, epithelial cell lines (see, for example, ref. 3) and in many primary epithelial cultures, and has been assumed to be an artefact of tissue culture conditions, or even of selection of a rare stem cell type². However, our findings with parietal endoderm suggest that the co-expression of vimentin and cytokeratin filaments may be a common property of epithelial cells that can survive as single cells, in particular those cell lines which are easily dispersed into single cells during tissue culture, and cells which disseminate *in vivo* from a compact epithelial tissue. Recent observations in tissue culture support this hypothesis. For example, in primary keratinocyte cultures, vimentin expression is preferentially seen at the free margins of the stratifying colonies; and in a cell line (SVK14) which can sporadically develop vimentin-positive cells at late passage, vimentin is seen only in rare solitary cells and not in the normal closely packed colonies (E.B.L., unpublished data).

The ability to survive as an individual epithelial cell without being part of a cohesive tissue could be a significant difference between those metastatic carcinomas where the cells invade as solid cords or groups, and those where the cells disperse as single cells into the body fluids. Vimentin has been reported in carcinoma cells growing in suspension in ascites or pleural effusions⁶ but is absent from solid metastases of similar and other carcinomas^{6,19,29,30}. Our finding that PE cells normally co-express vimentin and cytokeratins also raises the possibility that other cells which migrate away from simple epithelia during tissue morphogenesis may, at least for a time, synthesize both classes of intermediate filaments. If this is the case, the appearance of metastatic cells expressing both cytokeratin and vimentin filaments may be interpretable in terms of reversion to an embryonic behaviour pattern.

After this work was completed, we learnt that Dr E. Lehtonen (University of Helsinki) has now also observed immunofluorescent staining of PE cells with anti-keratin and anti-vimentin antisera (personal communication).

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Monoclonal suppressor T-cell factor displaying V_H restriction and fine antigenic specificity

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The production of stable T-cell clones is essential for the study of T-cell-derived, specific immunoregulatory products and of specific T-cell receptors. T-cell clones have been established by radiation leukaemia virus (RadLV)-induced transformation of suppressor T lymphocytes specific for hen egg white lysozyme (HEL). We report here that culture supernatant obtained from these T-cell clones can, when injected into mice, specifically suppress the anti-HEL antibody response. This monoclonal T-cell product suppresses the antibody response induced by HEL and human lysozyme, but not that induced by ring-necked pheasant egg white lysozyme (REL), thus displaying fine antigenic specificity probably restricted to an epitope involving phenylalanine at amino acid residue 3, present in the N-terminal region of HEL and shared by human lysozyme but absent in REL. The suppression induced by this monoclonal T-cell product is restricted by both *H-2* and *Igh-1* genes whereas anti-HEL antibodies bearing a predominant idiotype are induced in all mice strains tested, irrespective of their *H-2* haplotype or *Igh-1* allotype¹.

HEL is a well characterized monomeric protein and the cellular interactions involved in the regulation of the anti-lysozyme response have been extensively studied². Moreover, the amino acid sequence of HEL and several related lysozymes is known, allowing fine specificity analysis of HEL-specific immunoregulatory T cells and their products.

Intraperitoneal (i.p.) HEL-CFA priming in mice of *H-2*^b haplotype, genetically nonresponder to HEL³, induces antigen-specific, I-J⁺ suppressor T cells⁴. The procedure we have fol-

lowed to establish HEL-specific T-cell lines by RadLV-induced transformation⁵ of this cell population has been described elsewhere^{6,7}. Briefly, suppressor T cells from HEL-primed C57BL/6 (*H-2*^b) mice have been enriched by a sequential positive selection on anti-mouse immunoglobulin and HEL-coated plates. No attempts were made to determine the degree of enrichment in HEL-specific cells. This enriched suppressor T-cell population (80% I-J⁺) has been infected *in vitro* with RadLV/*nu*₁ virus collected from culture supernatants of the BALB/*nu*₁ cell line⁸, and infected cells were injected into sublethally irradiated syngeneic recipients. Within 4–6 months, 30% of the injected mice developed large thymomas which were analysed for T-cell markers by immunofluorescence. Among the six thymomas tested, one showed the expected surface markers for suppressor T cells (Thy 1.2⁺, Lyt 2⁺, I-J⁺, sIg⁺) and cell-free extracts obtained from this lymphoma showed HEL-specific suppressive activity in a T cell-dependent lymph node proliferative assay. A cell line (LH8) was then established *in vitro* from this lymphoma. Culture supernatants from the LH8 cell line demonstrated HEL-specific suppressive activity of T-cell proliferation⁶.

The LH8 cell line was cloned by limiting dilution on peritoneal macrophage feeder layers and culture supernatants from individual clones and the parental LH8 T-cell line were tested for suppressive activity (Fig. 1). BDF1 mice were injected i.p. with 100 µg of HEL-CFA and developed anti-HEL plaque-forming cells (PFC), as measured 8 days later in the parathymic lymph nodes. Culture supernatant from LH8 cell line or from

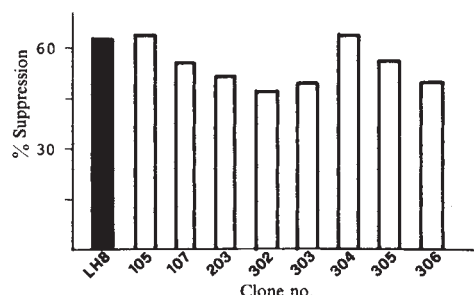


Fig. 1 Suppressive activity of clones derived from LH8 T-cell lymphoma line. (C57BL/10×DBA/2)_F₁ (BDF1) mice (5 mice per group) were injected i.p. with 100 µg HEL in CFA and anti-HEL PFC responses measured 8 days later in the parathymic lymph nodes, as described elsewhere⁹. Briefly, cell suspensions from parathymic lymph nodes (PT-LN) were sequentially teased through coarse and fine mesh screens into cold Eagle's minimum essential medium (Gibco). The cell suspensions were washed once, resuspended in cold medium and assayed for haemolytic plaque (PFC) formation using the Cunningham and Szenberg technique. Lysozyme was coupled to sheep erythrocytes (SRBC) by the mannitol modification of the 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (EDCI) coupling technique. SRBC were washed three times in saline, once in mannitol-saline (0.35 M mannitol–0.01 M NaCl) and resuspended at a 10% concentration in mannitol-saline. One ml of 10% SRBC was reacted with 0.1 ml of lysozyme (1 mg ml⁻¹ in distilled water) for 10 min on a rotator. Then 0.1 ml of EDCI in mannitol-saline (100 mg ml⁻¹) was added, the reaction was continued for an additional 30 min and finally the cells were washed three times with medium. Rabbit anti-mouse immunoglobulin serum was used at a final dilution of 1:400 to detect developed PFC. Fresh guinea pig serum, adsorbed with erythrocytes, was used as a source of complement at a final dilution of 1:64. LH8 cells were cloned by limiting dilution in microtitre plates (Nunc) containing 2×10⁴ adherent syngeneic peritoneal cells as a source of macrophages. LH8 cells were seeded at 0.3 cells per well and growth was observed after 2 weeks with >80% cloning efficiency. Culture supernatant from LH8 cell line or from the indicated clones was injected i.v. (1 ml per mouse) at the same time as antigen priming. Columns represent suppression of the control response obtained in mice not injected with culture supernatant (12,579 developed anti-HEL PFC per 10⁶ parathymic lymph node cells).

independent clones was injected intravenously (i.v., 1 ml per mouse) at the same time as antigen priming. The results in Fig. 1 demonstrate that a single injection of culture supernatant from any clone tested is able to suppress the anti-HEL PFC response. Clone LH8-105 was selected for further studies. The surface phenotype of clone LH8-105 cells, as determined by indirect immunofluorescence, was Thy 1.2⁺, Lyt 2⁺, I-J⁺, sIg⁻. Titration of culture supernatant from clone LH8-105, as assessed by suppression of *in vivo* antibody response or *in vitro* T-cell proliferation, demonstrated suppressive activity up to 10⁻² dilution (Fig. 2).

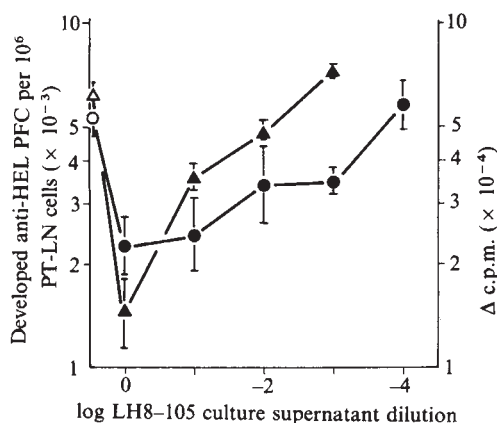


Fig. 2 Titration of LH8-105 culture supernatant suppressive activity. BDF1 mice (5 mice per group) were injected i.p. with 100 μ g HEL-CFA and developed anti-HEL PFC responses measured 8 days later. Values shown are the geometric mean PFC responses obtained in mice not injected with culture supernatant (○) or injected with culture supernatant from clone LH8-105 (●). Alternatively, BDF1 mice were immunized at the tail base with 10 μ g per mouse HEL-CFA and inguinal and periaortic lymph node cells cultured with 100 μ g ml⁻¹ HEL (Δ), as described previously⁶. Culture supernatant from clone LH8-105 (▲) was added to cultures at the indicated dilutions. Values are the geometric mean of net c.p.m. from triplicate cultures. Bars represent s.e.m.

Previous results had shown that injection of culture supernatant from the LH8 T-cell line was able to suppress the anti-lysozyme PFC response of mice primed with HEL whereas the response to REL was unaffected⁹. These results are confirmed and extended, at the clonal level, by the experiment shown in Table 1. BDF1 mice were immunized i.p. with HEL, REL or human lysozyme (100 μ g per mouse) and the anti-lysozyme PFC responses were measured 8 days later in the parathymic lymph nodes. Injection of LH8-105 culture supernatant induced suppression of anti-lysozyme PFC responses in mice primed with HEL and human lysozyme, but not in mice primed with REL. Therefore, the LH8-105 suppressor T-cell factor can discriminate between the closely related HEL and REL. On the other hand, this suppressive factor suppresses the anti-lysozyme response induced by human lysozyme to the same extent as that induced by HEL. These two lysozymes differ at almost 50% amino acid residues but they share an antigenic epitope, involving phenylalanine at position 3, that is critical for inducing suppression¹⁰.

The presence of a suppressive determinant common to HEL and human lysozyme despite the differences between these two lysozymes, may explain the high HEL-human lysozyme cross-reactivity in the immunosuppression exerted by HEL-specific monoclonal suppressor T-cell factor. The N-terminal regions of HEL and human lysozyme are identical at amino acid residues 1-9, except for glycine at position 4 in HEL which is replaced by glutamic acid in human lysozyme. REL has tyrosine instead of phenylalanine at position 3, which may explain the lack of LH8-105 suppressor T-cell factor activity in REL-primed mice. Therefore, the LH8-105 suppressor T-cell factor is able to discriminate between the closely related HEL and REL whereas it suppresses to the same extent the anti-lysozyme responses induced by HEL and human lysozyme, thus displaying fine antigenic specificity probably restricted to the N-terminal portion of the lysozyme molecule. On the other hand, there is very high cross-reactivity of PFC responses between HEL and REL but these show very low cross-reactivity with human lysozyme, indicating that suppressor T-cell and antibodies display different specificities.

Table 1 Fine antigenic specificity of LH8-105 suppressor T-cell factor

LH8-105 injection	Priming	Developed anti-lysozyme PFC per 10 ⁶ PT-LN cells			% Suppression of the specific response*	% Cross-reactivity at the level of PFC responses		
		Anti-HEL	Anti-REL	Anti-human lysozyme		HEL	REL	Human lysozyme
-	HEL-CFA	17,137 (1.30)	14,613 (1.40)	1,780 (1.14)		100	85	10
+	HEL-CFA	6,095 (1.18)	3,852 (1.26)	315 (1.51)	65	100	63	5
-	REL-CFA	11,010 (1.49)	17,085 (1.57)	280 (1.56)		64	100	2
+	REL-CFA	14,305 (1.20)	18,591 (1.37)	145 (1.23)	0	77	100	1
-	HUL-CFA	217 (2.58)	114 (1.96)	983 (1.64)		22	12	100
+	HUL-CFA	23 (1.84)	39 (1.67)	265 (1.33)	73	9	15	100

BDF1 mice (5 mice per group) were injected i.p. with 100 μ g HEL, REL or human lysozyme in CFA and PFC responses measured 8 days later in the parathymic lymph nodes. Culture supernatant from LH8-105 cell clone (1 ml per mouse) was injected for three consecutive days and the first injection was given on the same day as antigen priming. PFC values are expressed as the geometric mean and the numbers in parentheses represent a factor by which the mean should be multiplied or divided to give one standard error.

* Per cent suppression refers to the homologous anti-lysozyme PFC response.

Table 2 *Igh-1*^b restriction of LH8-105 suppressor T-cell factor activity

Mice	<i>H-2</i>	<i>Igh-1</i>	Developed anti-HEL PFC per 10 ⁶ PT-LN cells		% Suppression
			-	+	
(C57BL/10 $\times$ DBA/2)F ₁	<i>b/d</i>	<i>b/c</i>	10,127 (1.38)	1,361 (2.11)	87
C3H	<i>k</i>	<i>j</i>	5,949 (1.45)	7,076 (1.36)	0
(C57BL/6 $\times$ C3H)F ₁	<i>b/k</i>	<i>b/j</i>	9,357 (1.18)	2,518 (1.33)	73
(C3H.SW $\times$ C3H)F ₁	<i>b/k</i>	<i>j/j</i>	16,137 (1.20)	20,140 (1.17)	0

Mice were injected i.p. with 100 μ g HEL and PFC responses measured 8 days later. Culture supernatant from clone LH8-105, diluted 1:50, was injected on three consecutive days. PFC values are expressed as the geometric mean and the numbers in parentheses represent a factor by which the mean should be multiplied or divided to give one standard error.

The HEL-specific suppressor T-cell factor produced by the LH8-105 cell clone, established from suppressor T cells on nonresponder C57BL/6 ($H-2^b$, $Igh-1^b$) mice, has been routinely tested for suppression in semi-syngeneic (C57BL/10 $\times$ DBA/2) F_1 ($H-2^{b/d}$, $Igh-1^{b/c}$) mice, responder to HEL. Previous studies had demonstrated that the suppressive activity of the factor produced by LH8 cells, from which clone LH8-105 was derived, is restricted by genes of the $H-2$ complex determining the I-J specificity⁹. To assess whether the activity of the LH8-105 monoclonal suppressor T-cell factor is V_H -restricted, mice of different $Igh-1$ haplotype were primed i.p. with HEL-CFA (100 μ g per mouse) and the anti-HEL PFC response was measured 8 days later in the parathymic lymph nodes. The results given in Table 2 show that the anti-HEL antibody response induced in C3H ($H-2^k$, $Igh-1^l$) and in (C3H.SW $\times$ C3H) F_1 ($H-2^{b/k}$, $Igh-1^{l/l}$) mice is not suppressed by injection with LH8-105 culture supernatant whereas suppressive activity is very evident in (C57BL/10 $\times$ DBA/2) F_1 ($H-2^{b/d}$, $Igh-1^{b/c}$) and in (C57BL/6 $\times$ C3H) F_1 ($H-2^{b/k}$, $Igh-1^{b/l}$) mice. As C57BL/6 and C3H.SW are $Igh-1$ congenic mice, the interaction between this HEL-specific suppressor T-cell factor and its cellular target seems to be restricted by $Igh-1$ genes. This restriction was unexpected because the predominant idiomotype displayed by anti-HEL antibodies is

expressed on most HEL-reactive antibodies in all strains tested, irrespective of their $Igh-1$ allotype or $H-2$ haplotype¹. The failure of LH8-105 T-cell to suppress across an $Igh-1$ allotype barrier may indicate that this suppressive factor interacts with its target by recognizing also determinants linked to the $Igh-1^b$ complex.

Thus, we have demonstrated a dual restriction of this monoclonal suppressor T-cell product, as opposed to the lack of restriction in the induction of idiomotype-positive anti-HEL antibodies.

Moreover, the existence of different specificity repertoires between suppressor T cells and B cells directed against the same antigen has been confirmed at the clonal level. Taken together, these results indicate that genes coding for the variable region of this T-cell product and V genes used by B cells to produce idiomotype-positive anti-HEL antibodies may not be related, as also suggested, in other systems, by nucleic acid hybridization studies^{11,12}.

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Evidence for host-cell selection of influenza virus antigenic variants

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Extensive antigenic variability and a capricious epidemiology are characteristics of influenza A and B viruses of man. The haemagglutinin (HA) undergoes frequent and progressive antigenic drift as a result of selection, under immunological pressure, of viruses possessing alterations in the amino acid sequences at specific sites in the molecule¹. Here we present evidence for an additional selection mechanism for antigenic variants of influenza virus that depends on differing host cell tropisms of virus subpopulations. These studies were initiated after earlier observations of the occurrence of a marked degree of antigenic variation during passage of laboratory strains of influenza virus in eggs and cell cultures (J.C.J., in preparation). We have now shown that cultivation of influenza B viruses in eggs selects subpopulations which are antigenically distinct from virus from the same source grown in mammalian cell cultures. As antigenic characterization of influenza virus strains for epidemiological purposes² and for the preparation of influenza vaccines³ conventionally relies on the cultivation of virus in eggs, our findings may have important practical implications for vaccine design and efficacy.

Table 1 shows haemagglutination inhibition (HI) reactions of six influenza B viruses representative of 27 strains, isolated

and passaged in eggs or canine kidney cell cultures (MDCK)^{4,5}, from specimens collected during a school outbreak of influenza in 1982 (J.S.O. *et al.*, in preparation). The virus preparations were tested against anti-HA monoclonal antibodies prepared against influenza B virus cultivated in eggs or MDCK cells as described in Table 1 legend. Three patterns of serological reactivity were apparent. Monoclonal antibody 195 reacted to high titres with all the influenza B viruses tested. Monoclonal

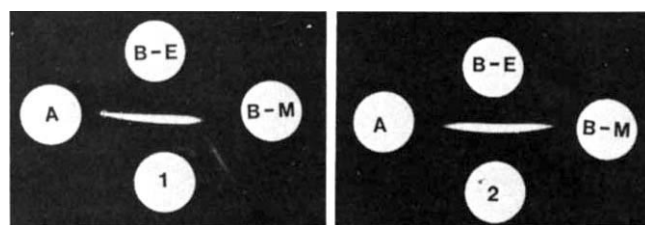


Fig. 1 Double immunodiffusion reactions of rabbit antiserum prepared against the purified haemagglutinin of B/Singapore/222/79 virus which was isolated and passaged in eggs alone. Antisera: well 1 contained unadsorbed serum; well 2 contained serum which had been adsorbed with concentrated B/England/145/82 virus which had been isolated and passaged in MDCK culture only. Antigens: wells marked A contained concentrated purified egg-grown influenza A/Hong Kong/1/68 (H3N2) virus; wells B-E contained concentrated, purified, egg-brown B/England/145/82 virus which had been isolated in MDCK cultures and subsequently passaged twice in eggs; wells B-M contained concentrated, purified B/England/145/82 virus isolated and passaged in MDCK culture only. Spur formation between the precipitin lines given by B-M and B-E is evident. After adsorption of the serum with antigen B-E, no precipitin lines were detected with either B-M or B-E antigen (data not shown). The pattern of precipitin reactions was not affected by preliminary absorption of the serum with concentrated, egg-brown A/Hong Kong/68 (H3N2) virus, which would be expected to remove antibody to 'host' antigen of egg origin.

antibody 209 reacted with all the viruses that had been isolated and subsequently passaged in MDCK cultures but not with those that had been cultivated in eggs at any stage in their passage histories. Antibody 238 reacted only with viruses which had at least one passage in eggs and with none of the viruses that had been passaged in MDCK cultures alone. The mutual exclusivity of the reactions of antibodies 209 and 238 with the 27 virus preparations was striking.

Viruses having different passage histories in eggs or cell cultures would be expected to react differently with monoclonal antibodies if these were directed against antigens present as carbohydrate moieties^{6,7} derived from host-cell membranes and covalently linked to the haemagglutinin molecule. However, this does not explain our findings. None of the monoclonal antibodies gave HI reactions which were entirely determined by the last culture system in which the test virus was grown. Moreover, the reactions were influenza B-specific and were not affected by absorbing the antibodies with homogenates of chorioallantoic membrane or MDCK cells.

Further investigations were carried out with plaque-purified viruses derived from B/England/145/82. Virus which had been isolated and passaged twice on MDCK cultures, with an infectivity titre of $10^{7.7}$ plaque-forming units (PFU) ml^{-1} in MDCK cells and $10^{3.5}$ egg infective dose (EID) ml^{-1} by the allantoic route in embryonated hen eggs, was seeded at high dilutions on MDCK monolayers; 200 well separated plaques were collected and eluted into small volumes of medium (see legend to Table 2). The plaque eluates were inoculated undiluted into further MDCK cultures and also into the allantoic cavities of fertile eggs. A high proportion of the eluates successfully infected MDCK cells whereas only 5 of the 200 plaque eluates produced growth in eggs. All 190 plaque-derived viruses collected from MDCK cultures and the five plaque-derived viruses which grew in eggs reacted to high titres with monoclonal

antibody 195. Monoclonal antibody 238 reacted with each of the five viruses generated in eggs but failed to react with the 190 viruses prepared in MDCK cultures. The converse pattern of reactions was obtained with monoclonal antibody 209, which reacted with the MDCK collections but failed to react with the egg collections.

The differing serological reactivities of the two groups of plaque-derived viruses with antibodies 238 and 209 were not simply a direct reflection of the last substrate in which the virus had been passaged. When the five viruses prepared in eggs were given a further passage in MDCK cultures, the HI reactions of the MDCK collections were identical to those of the parental allantoic fluids, that is, all of them reacted with antibody 238 but none reacted with antibody 209.

Plaque isolation experiments were also carried out with B/England/145/82 virus initially isolated in MDCK cells and further passaged once in eggs. The infectivity titre of this virus was $10^{8.5}$ PFU ml^{-1} in MDCK cultures and $10^{9.7}$ EID₅₀ ml^{-1} on allantoic inoculation of eggs. Twenty-four plaques were picked; the progeny of all these plaques grew in the allantoic cavity and in MDCK cultures. All collections from eggs or MDCK cells reacted with monoclonal antibody 238 but not with antibody 209.

The clear antigenic differences between the influenza B viruses described above which differed in their reactions with monoclonal antibodies, were also detectable with polyclonal sera. The virus preparations listed in Table 1 were tested with post-infection ferret antisera to egg-grown B/England/145/82 and B/Singapore/222/79 viruses. The HI titres were 2–40-fold higher (mean 10-fold higher) for viruses which had been passaged on MDCK cells alone than for those that had been passaged at least once in eggs. The HI reactions of ferret antisera with plaque-purified B/England/145/82 viruses were also investigated (Table 2). Viruses from the same two plaques were

Table 1 Haemagglutination inhibition reactions of anti-HA monoclonal antibodies with influenza B viruses of different isolation and passage histories

Isolation and passage history of B/England/82 viruses		Monoclonal antibodies to:		
Specimen no.	Cell substrate for virus isolation* and passage†	Egg-grown B/Oregon/5/80 virus		MDCK cell-grown B/England/145/82 virus
		195	238	209
145	M*-M†	25,600	<	320
	M-M (×3)	12,800	<	320
	M-Al	38,400	9,600	<
	M-Al (×3)	12,800	9,600	<
	M-Al-M (×3)	12,800	9,600	<
222	M-M	≥6,400	<	160
	M-Al	≥6,400	6,400	<
	Am-Al	4,800	6,400	<
	Am-Al-M (×3)	6,400	6,400	<
	M-M	≥6,400	<	40
272	M-Al	6,400	6,400	<
	M-Al (×3)	6,400	6,400	NT
	Am-Al	3,200	4,800	<
	Am-Al-M (×2)	NT	12,800	<
	M-M	≥6,400	<	>1,280
203	M-Al	≥6,400	≥6,400	<
	M-M	≥6,400	<	>1,280
290	M-Al	≥6,400	≥6,400	<
	M-Al-M (×3)	25,600	≥6,400	NT
183	M-M	≥6,400	<	160
	M-Al	6,400	4,800	<
	B/Oreg/5/80 egg-grown	6,400	4,800	<

M indicates isolation or passage in MDCK cell cultures; Al indicates passage in the allantoic cavity of embryonated eggs; Am, passage in the amniotic cavity of embryonated eggs. Numbers in parentheses indicate number of serial passages. <, HI titres <1:50 or, for antibody 209, <1:10. NT, Not tested. Monoclonal antibodies 195 and 238 were prepared against purified, egg-grown B/Oregon/5/80 virus by standard techniques¹⁷⁻¹⁹. Monoclonal antibody 209 was prepared by immunizing mice with B/Eng/145/82 virus isolated and cultivated in MDCK cells. Mice were immunized intraperitoneally with ~3,000 HA units of purified influenza virus and approximately 12 weeks later boosted intravenously with purified virus. Spleens were removed 4 days later and spleen cells fused to SP20Ag14 myeloma cells. Hybridomas secreting specific anti-HA antibodies were selected using HI and ELISA techniques and cloned in soft agar. Ascitic fluids were collected from mice injected intraperitoneally with 10^7 hybridoma cells. Additional monoclonal antibodies (124, 146, 162) prepared against egg-grown B/Oregon/80 virus showed similar patterns of HI reactivity to antibody 238, reacting with virus passaged at least once in eggs and not with virus grown exclusively in cell culture.

Table 2 Growth of plaque-purified clones of B/England/145/82 virus in MDCK cultures and fertile eggs and haemagglutination inhibition reactions of progeny virus with monoclonal antibodies and post-infection ferret sera

Substrate	Proportion of clones showing growth in MDCK cultures and eggs* (no. of plaques producing viral growth/no. tested)	HI reactions of progeny virus with monoclonal antibodies:			HI reactions of progeny virus with post-infection ferret sera†				
		No. reacting/no. tested (range of titres)			F ₁	F ₂	F ₃	F ₄	F ₅
		195	238	209					
MDCK culture	194/200 (97%)	$\frac{190}{190}$ (2,560– $\geq$ 12,800)	$\frac{0}{190}$ (all <100)	$\frac{112}{112}$ (40–640)	a 320 b 160	960 640	480 320	320 120	480 320
Egg	5/200 (2.5%)	$\frac{2}{5}$ (9,600– $\geq$ 12,800)	$\frac{2}{5}$ (all $\geq$ 12,800)	$\frac{0}{5}$ (all $\leq$ 20)	a 40 b 20	120 60	80 60	30 20	120 60

* Agar cores overlaying individual plaques in MDCK cultures were taken up with Pasteur pipettes and the virus eluted into 0.3-ml volumes of phosphate-buffered saline (PBS). To test growth in MDCK cells, 0.15 ml of each eluate was inoculated into cultures grown in 12-well flat-bottomed tissue culture plates, each well being 4.5 cm² in area and containing 1.5 ml medium (minimal essential medium, 2% NaHCO₃). The cultures were incubated for 72 h at 36 °C in an atmosphere of 5% CO₂. The medium was collected and virus growth detected by haemagglutination assay with chick erythrocytes. To test growth in eggs, 0.15 ml of eluate was inoculated into the allantoic cavity of 10-day fertile eggs. Allantoic fluids were tested for haemagglutinin after incubation for 72 h at 35 °C.

† Sera collected 14 days post-infection with B/England/145/82 virus. Ferrets F₁, F₂ and F₃ were infected with virus isolated and grown in eggs; ferrets F₄ and F₅ were infected with virus isolated and grown in MDCK cell cultures. Data are shown for the progeny of two plaques (a and b) picked from MDCK cultures and grown either in MDCK cells (upper half of table) or in eggs (lower half of table).

inoculated onto MDCK cells and into eggs and the progeny tested with the ferret antisera. The HI titres against virus prepared in MDCK cultures were 4–11-fold (mean 7-fold) higher than the titres against virus prepared in eggs.

Large differences were also observed in the frequency and titre of HI antibody in the sera of unvaccinated children and young adults to two influenza B viruses (grown in eggs or MDCK cells) from the same clinical specimen or from the same MDCK plaque (Table 3). Many sera showed high HI titres ($\geq$ 1,280) with B/England/222/82 virus passaged exclusively on cell cultures and were negative (titre <10) when tested with corresponding egg-grown virus. None of the 288 serum samples tested exhibited a higher antibody titre when tested against egg-grown virus compared to MDCK-grown virus.

Further evidence of antigenic differences between B/England/145/82 virus preparations with different passage histories were detected in double immunodiffusion tests⁸ using specific immune anti-HA sera prepared in rabbits (Fig. 1) which were immunized with HA derived from a reference strain of influenza B virus—B/Singapore/222/79 grown in eggs. The anti-HA serum gave clear precipitation reactions with viruses prepared in MDCK cultures and in eggs; however, spur formation at the junction of the precipitin lines produced by the two antigens indicated that the antiserum reacted with HA determinants of the egg-grown virus which were not present on the MDCK-grown virus. After absorption with concentrated MDCK-grown B/England/145/82 virus a precipitin line was detected with egg-grown virus only. In contrast (data not shown), serum absorbed with egg-grown virus failed to react with either antigen.

In experiments to investigate any biological differences between the antigenically distinct subpopulations, the range of erythrocytes agglutinated by the viruses was compared. Erythrocytes may be used as a model for cell-receptor specificity for influenza virus. The preparations of B/England/145/82 and B/England/222/82 listed in Table 1 were tested with erythrocytes of chicken, turkey, man (group A, AB and O cells), rhesus monkey, guinea pig, sheep, rat and horse. The viruses, irrespective of passage history, agglutinated the cells of these

species to similar titres, except for sheep, horse and rat erythrocytes. For cells from the latter three species, high agglutination titres were detected with virus which had been isolated or passaged in eggs but no agglutination was detected for virus passaged in MDCK cultures alone.

Our findings provide strong evidence that influenza B virus isolated from clinical specimens and grown in MDCK cultures comprises at least two biologically and antigenically distinct subpopulations. A minor proportion of the MDCK-grown virus is capable of growth in the allantois of fertile eggs without previous adaptation, and possesses HA which is antigenically distinct from the majority of the virus which grows poorly or not at all in the allantoic cavity.

Our studies are not unique in providing evidence of distinct subpopulations of virus within a single strain. Other reports^{9,10} have documented biological or antigenic heterogeneity among populations of influenza A virus cultivated in eggs. However, we have described for the first time evidence of strong selective pressure for antigenic variants exerted by the cultivation of influenza viruses in eggs. A possible explanation for co-variance between growth in eggs and antigenic character of influenza B virus, consistent with the observation of differences in the specificity of cell- and egg-grown influenza B virus in haemagglutination reactions with erythrocytes of different species, is that one of the antigenic sites of the HA molecule is structurally and functionally related to the binding site for cell-surface receptors. Analysis of influenza A haemagglutinin indicates that the epitopes¹¹ with which monoclonal antibodies react are clustered into five independent antigenic sites in the three-dimensional structure of the molecule^{12,13} and, of these, site B seems to be topographically closest to the probable binding site for cell-surface receptors. The relevance of our findings to the known⁸ ability of influenza virus to vary in avidity to anti-HA antibody and in susceptibility to nonspecific inhibitors of haemagglutination, requires further study.

Thus, serological studies of the antigenic characteristics of virus strains for epidemiological purposes or studies of cellular¹⁴ or humoral parameters⁸ of immunity in man that are performed exclusively with egg-grown virus, may give misleading or at

Table 3 Haemagglutination inhibiting antibody in human sera to influenza B viruses with different passage histories

Virus origin and passage history	% Of sera with stated HI antibody titres:							
	Children*				Young adults†			
	$\geq$ 10	$\geq$ 40	$\geq$ 160	GMT	$\geq$ 10	$\geq$ 40	$\geq$ 160	GMT
B/England/222/82 M-M	83	76	57	110	100	100	100	590
Am-AI	20	8	2	<10	20	2	1	<10
B/England/145/82 M-M	58	24	4	15	ND	ND	ND	ND
M-AI	7	3	1	<10	ND	ND	ND	ND

* Sera collected in 1982 from 181 unvaccinated children aged 2–8 yr attending hospital clinic for treatment or investigation.

† Sera collected in October 1982 from 107 university students aged 18–22 yr were treated with receptor-destroying enzyme⁸ before testing. ND, Not determined. GMT; geometric mean titre.

best incomplete data. Our results also raise questions about the choice of substrate for the preparation of inactivated influenza vaccines. Antigenic differences between egg-grown influenza virus and virus shed by infected persons may be one of the several factors which contribute to the limited efficacy of inactivated influenza vaccines^{3,15,16}. Finally, non-immune selection of antigenically variant viruses in nature may occur by the selective growth of viruses in different target cell types in the respiratory tract of man and other natural hosts and thereby provide a further potential mechanism for antigenic 'drift'.

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Immunoreactive β -endorphin in sheep ovary

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Although the ovarian production of sex steroids is of obvious physiological importance, recent studies suggest that peptides such as oxytocin, relaxin and inhibin are also synthesized in the ovary¹⁻¹¹. We report here the presence of immunoreactive (ir) β -endorphin, ir-adrenocorticotrophic hormone (ACTH) and presumptive high molecular weight forms of both in extracts of sheep ovary, consistent with ovarian production from a common precursor. Our findings suggest that β -endorphin and ACTH are produced and secreted by the follicular cells, and that their production may be related to the oestrous cycle.

Ovaries from oestrous and anoestrous ewes were extracted as described in Fig. 1 legend. ACTH and β -endorphin content was determined by radioimmunoassays using rabbit antisera recognizing part or all of the amino acid sequence 18-24 of synthetic ACTH (1-39) (antiserum R1-3) and part or all of the amino acid sequence 17-26 of synthetic human β -endorphin (1-31) (antiserum R56); sensitivity of the ACTH and β -endorphin assays was routinely 2 pg and 5 pg per tube, respectively. Serial dilutions of the ovarian extracts, and of the fluid from large ovarian follicles (> 5 mm) from oestrous ewes, were parallel with standard curves of synthetic peptides in both radioimmunoassay systems.

The ovarian extracts were characterized further by Sephadex G-50 gel chromatography (Fig. 1). The chromatographic profiles of ir- β -endorphin showed three distinct peaks—a minor

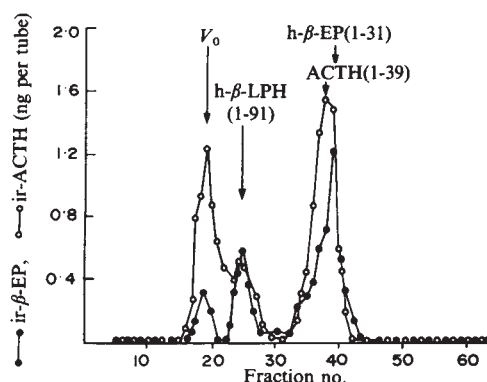


Fig. 1 Chromatographic profiles of immunoreactive β -endorphin (ir- β -EP, ●) and ir-acth (○) from ovarian extracts. Sheep ovaries from the peri-ovulatory period were collected in 5 ml of 0.1 M HCl at 4°C. Extracts were prepared by boiling for 15 min, cooling on ice and homogenization with a Polytron (Brinkmann Instruments speed setting 3, 3 × 3-s bursts). Homogenates were centrifuged (10,000g, Sorvall RC-5, DuPont) for 20 min at 4°C. The supernatant was removed, lyophilized and reconstituted in 10 ml of 1% formic acid, and a 3-ml sample applied to a Sephadex G-50 fine column (1.9 × 54 cm), equilibrated and eluted at room temperature with 1% formic acid containing bovine serum albumin (0.2 mg ml⁻¹). Fractions (2 ml) were collected, lyophilized, reconstituted in 1 ml assay buffer and assayed with β -endorphin antiserum (R56) and ACTH antiserum (R1-3). The column was calibrated with blue dextran (void volume, V_0), purified human β -LPH (gift of Dr P. Lowry), synthetic human β -endorphin (1-31) (Peninsula) and purified human ACTH(1-39) (MRC 74/555). For radioimmunoassay ¹²⁵I- β -endorphin tracer was prepared by iodination of human β -endorphin followed by Sephadex G-25 chromatographic separation; ¹²⁵I-acth was from International CEA. Rabbit antiserum raised against human β -endorphin (R56) is specific for the decapeptide β -LPH(78-87), and thus did not recognize [Met]enkephalin, [Leu]enkephalin ACTH(1-39), corticotropin-like immunoreactive peptide (CLIP), α -melanocyte-stimulating hormone (α -MSH); α - or γ -endorphin, but cross-reacted on an equimolar basis with β -LPH, β -LPH(61-87), N -acetyl β -LPH(61-87), and N -acetyl β -endorphin. Rabbit antiserum (R1-3) against Synacthen (ACTH(1-24)) did not recognize ACTH(4-10), α -MSH, CLIP, human β -endorphin, [Met]enkephalin or [Leu]enkephalin. Details of the assays have been described previously^{13,15}.

peak in the void volume and two major peaks eluting in the same positions as 11,500 molecular weight (11.5K) β -lipotropin (β LPH) and synthetic 3.5K β -endorphin, with the latter peak being the more prominent. For ir-acth, two major peaks were found, one eluting at the void volume and the other co-eluting with human ACTH (1-39) (MRC 74/555), with a less prominent intermediate peak.

Although the chemical identities of the various peaks of ovarian ir- β -endorphin and ir-acth were not established, the profile of β -endorphin-like immunoreactivity on gel chromatography strongly suggests the presence of 3.5K β -endorphin, 11.5K β -LPH and a higher molecular weight species. The profile of ACTH-like immunoreactivity is similarly consistent with the presence of 4.5K ACTH, ~13.5K 'big ACTH', and a higher molecular weight form (or forms) which may include a common ACTH and β -endorphin precursor. The difference between the apparent abundance of high molecular weight forms of ir-acth and ir- β -endorphin, and in particular the marked shoulder observed for this species of ir-acth, suggests that an additional high molecular weight form of ir-acth (~22K) may occur in the sheep ovary, as has been reported in the pituitary¹².

On a quantitative basis, the amount of ir- β -endorphin in the sheep ovary approximates that in rat hypothalamus¹³; in both tissues, the content is two to three orders of magnitude less than that present in the pituitary. As such, ir- β -endorphin in the ovary seems unlikely to contribute substantially to circulating ir- β -endorphin; it is possible, however, that ovarian ir- β -

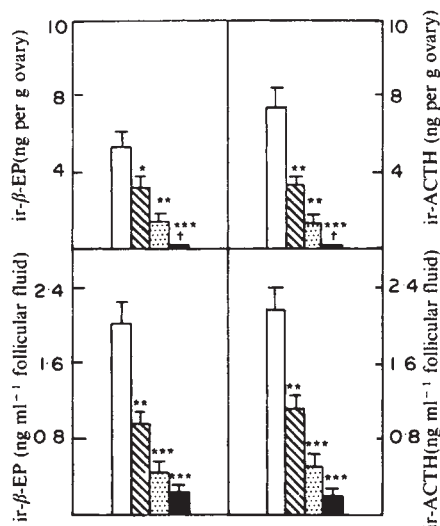


Fig. 2 Levels of ir- β -endorphin and ir-ACTH in sheep ovary and ovine follicular fluid. Ovaries (upper panel) from peri-ovulatory sheep (□), sheep in the luteal phase in the breeding season (▨) or sheep in the non-breeding season (▩) were extracted as described in Figure 1 legend. Ir- β -endorphin (left) and ir-ACTH (right) concentrations in these samples (and for comparison, those in corpus luteum extracts (■) of non-pregnant ewes) were determined. Aspirated ovarian follicular fluid (lower panel) from large follicles (>5 mm, □), small follicles (<4 mm, ▨) of oestrous ewes, follicles (~3 mm) from ewes in the luteal phase (▩) and follicles gathered in the non-breeding season (■), were collected and assayed for ir- β -endorphin and ir-ACTH. Values shown are mean $\pm$ s.e.m., ng ml⁻¹, $n=4$. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with levels in peri-ovulatory samples. † Not distinguishable from zero.

endorphin acts as a local modulator, perhaps affecting maturation or transportation of oocytes. A similarly local role has been proposed for ACTH and β -endorphin-related peptides in the male rat reproductive tract, recently reported by Tsong *et al.*¹⁴

As shown in Fig. 2, ovarian ir- β -endorphin levels may be related to the oestrous state of the animals. Significantly higher concentrations of ir- β -endorphin were found in ovaries removed in the peri-ovulatory period than in those removed from anoestrous ewes ($P<0.001$). In contrast, levels of ir- β -endorphin in corpus luteum extracts prepared in identical conditions were undetectable (<5 pg per tube). Levels of ir-ACTH in sheep ovaries closely paralleled those of ovarian ir- β -endorphin in all circumstances examined.

The concentration of ir- β -endorphin in ovarian follicular fluid seems to bear some relationship to the size of follicles collected from an individual animal. Figure 2 shows that at day 0 of the oestrous cycle, ir- β concentration in fluid from large follicles (>5 mm) was significantly higher than from smaller follicles (<4 mm). In contrast, fluid pooled from ovarian follicles (~3 mm) from anoestrous ewes contained no detectable levels of ir- β -endorphin or ir-ACTH.

The parallel changes in follicular size and follicular fluid ir- β -endorphin concentration prompted us to examine the possibility that follicular cells are a source of ir- β -endorphin. Figure 3 shows that β -endorphin immunofluorescence-positive cells were found after a 48 h *in vitro* monolayer culture of follicular cells from oestrous animals, while ir- β -endorphin and ir-ACTH were also clearly demonstrable in culture medium (Fig. 4). Higher levels of the two peptides were present in the medium conditioned by cells from larger follicles (>5 mm) than from smaller follicles (<4 mm) taken from the same ovaries. In contrast, neither β -endorphin-immunofluorescent cells nor detectable levels of ir- β -endorphin in the medium were found in follicular cell cultures prepared from anoestrous animals; similarly negative results were obtained in cultures prepared in

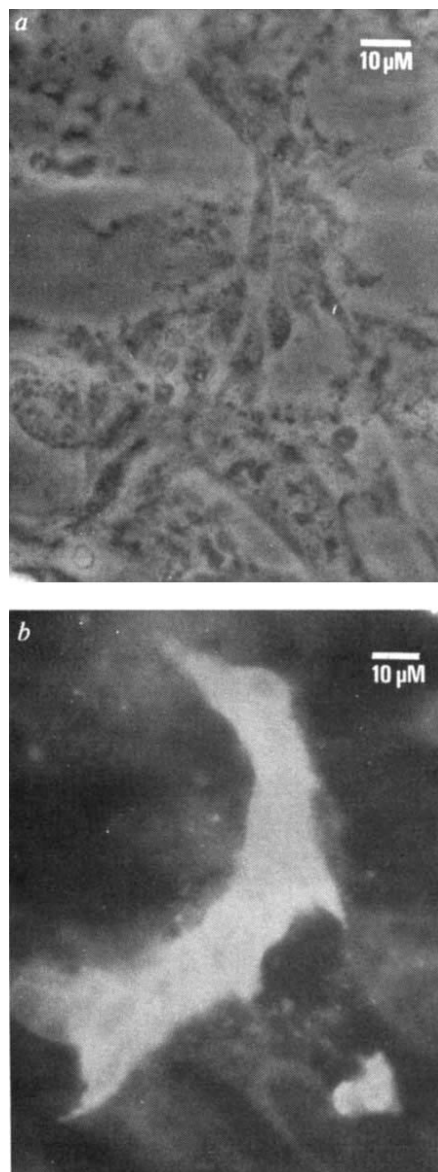


Fig. 3 Phase-contrast and indirect immunofluorescence microscopy of dissociated sheep ovarian follicular cells. *a*, Follicular cell culture at 48 h seen under phase-contrast; *b*, same field as in *a* with immunofluorescence-positive cells stained with β -endorphin antiserum (1:16). Scale bar, 10 μ m. Immunofluorescence staining was performed on cells cultured for 48–72 h on sterile glass coverslips as described in Fig. 4 legend. Before staining, cells were rinsed in phosphate buffer solution (PBS), fixed in Bouin's solution for 4 h at room temperature (RT) followed by 3×10 min PBS washes. Rabbit β -endorphin antiserum (R56) was applied at dilutions ranging from 1:8 to 1:64 in PBS with 0.25% Triton X-100 and 0.1 M lysine-HCl, pH 7.4, and incubated for 2 h at RT. Following 3×10 min PBS washes, fluorescein-conjugated sheep anti-rabbit IgG (Wellcome) was applied at 1:32 dilution, and further incubated for 30 min at RT. Coverslips were then washed with PBS for 30 min, and mounted in veronal-buffered glycerol, pH 8.6. Specificity of the staining reactions was demonstrated by the absence of specific staining with PBS, with normal rabbit serum, or with β -endorphin antiserum at 1:32 dilution pre-absorbed with an equal volume of 5 μ g ml⁻¹ synthetic human β -endorphin. Staining was unaffected by prior absorption with vasopressin, ACTH or [Met]enkephalin at concentrations of 50–100 μ g ml⁻¹. Not shown is equivalent immunofluorescence staining obtained with an anti- β -endorphin antiserum raised by Dr S. Yen (San Diego), provided by Dr John Oliver (Flinders Medical Centre), an antiserum which recognizes different determinants on the β -endorphin sequence¹⁶ from our in-house R56 antiserum. Indistinguishable immunofluorescence staining was also obtained with antisera to ACTH from Burroughs-Wellcome, and from Immunonuclear, Stillwater, Minnesota.

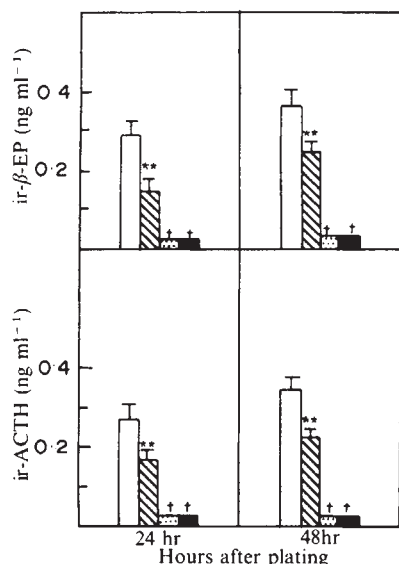


Fig. 4 Levels of ir- β -endorphin and ir-ACTH in the medium of sheep ovarian follicular cell culture. Large ovarian follicles (>4 mm, □) and small ovarian follicles (<4 mm, ▨) of oestrous ewes were isolated, and follicular cells collected with a fine loop of chromium-steel wire (30-gauge). The cells were washed twice in PBS and incubated in HEPES 199 with collagenase (1 mg ml⁻¹) at 37 °C for 20 min. The suspension was centrifuged (250g, 5 min) and the cell pellet rinsed twice with PBS, triturated with a siliconized pipette and plated at a density of 0.2×10^6 cells on glass coverslips in culture wells. Cell viability as ascertained by Trypan blue exclusion was >90%. The cells were grown in a humidified atmosphere of 5% CO₂/95% air at 37 °C in medium (M 199 HEPES, 5% horse serum, 5% fetal calf serum, 2 mM glutamine, 1% non-essential amino acids and 40 U ml⁻¹ penicillin). Media were collected after 24 or 48 h of plating, centrifuged for 2 min at 4 °C (Beckmann microfuge B) and the supernatant removed for radioimmunoassay. Recovery of added synthetic human β -endorphin (500 pg in 1 ml of medium) after 24 h co-incubation with the culture in newly replaced medium was $88 \pm 7\%$ (mean $\pm$ s.e.m., $n = 6$), taking into account peptide secreted from the cells. Similarly, recovery for human ACTH(1-39) (MRC 74/555) of equivalently added amounts in similar conditions was $82 \pm 6\%$. Values shown were mean $\pm$ s.e.m., ng ml⁻¹, $n = 5$. ** $P < 0.01$ compared with samples from large follicles. For comparison, cells from small follicles of anoestrous ovaries obtained at non-breeding season (▩), and cells from corpora lutea (■) of non-pregnant ewes were prepared and cultured in identical conditions; ir- β -endorphin and ir-ACTH in the culture media after 24 h and 48 h plating in both cultures were not detectable (ir- β -endorphin <5 pg per tube; ir-ACTH <2 pg per tube).

the same way from corpora lutea. This contrasts with recent findings that large amounts of oxytocin are contained in the ovine corpus luteum¹.

Ovarian levels of both ir- β -endorphin and ir-ACTH, and those in the medium from cultured follicular cells, appear related to the oestrous stage of the ewes. One interpretation of this finding is that follicular cell production of ir-ACTH and ir- β -endorphin, like that of ovarian steroids, is controlled by pituitary gonadotrophins, either directly or indirectly. We are now investigating the possible relationship between ovarian steroidogenesis and ovarian levels of ir-ACTH and ir- β EP.

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Novel clonal strains from adult rat anterior pituitary producing S-100 protein

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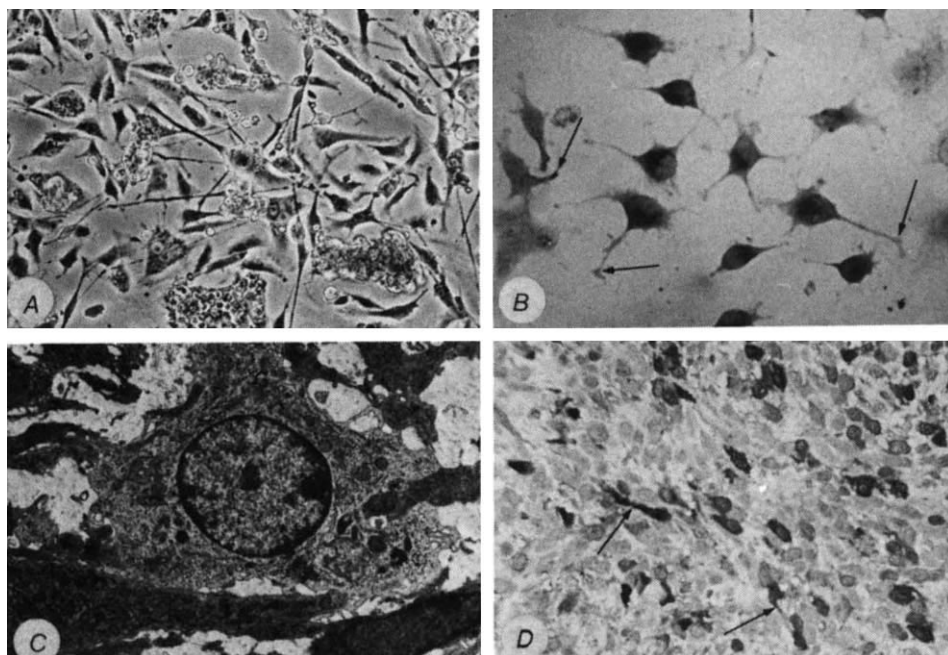
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S-100 protein, one of the unique proteins found in the nervous system^{1,2}, has recently been discovered unexpectedly in the rat anterior pituitary^{3,4}. Immunocytochemistry reveals that stellate, follicular and folliculostellate cells⁵, and marginal cells⁴ of the rat anterior pituitary contain this protein; however, as far as we know, there are no reports on the physiological role of this protein in the anterior pituitary. In the study reported here, three S-100 protein-producing clonal strains (JH-S3, JH-S8 and JH-S12) from adult rat anterior pituitaries were established by using the single cell-plating feeder layer method⁶. These new clonal strains reveal that the S-100 protein-producing cell is an independent cell type of the anterior pituitary. Both cultures and grafts of the JH-S3 cells stain immunocytochemically with anti-S100 protein IgG fraction. Moreover, the S-100 protein and conditioned medium of JH-S3 clonal cells both stimulate release of prolactin from prolactin-secreting clonal cells (1G4)⁷ *in vitro*.

It is generally believed that the S-100 protein is unique to the glial cell, particularly the astrocyte-like cell⁸. Recently, this unique brain protein was found in agranular cells: stellate, follicular and folliculostellate cells⁵, and also in the marginal cells of the rat anterior pituitary⁴. Different hypotheses have been proposed to explain the origin and role of follicular cells such as ACTH-secreting cells⁹⁻¹², stem cells¹³, dedifferentiated glandular cells¹⁴, supportive cells¹⁵ and phagocytic cells^{16,17}. Frémont and Ferrand¹⁸ postulate that the follicular-like cells are derived from peripheral epithelial cells. There are several possible hypotheses to explain the embryology of these cells: (1) they originate from the subependymal cells in the floor of the post-optic hypothalamus in the same manner as the pituicyte cell; (2) these cells originate from Rathke's pouch which is not considered a derivative of the stomodeal ectoderm but of the neuroectoderm of the caudal portion of the ventral bridge^{19,20}; and (3) multipotential cells (stem cells) of the anterior pituitary can differentiate into follicular cells.

Clonal strains were established by collecting anterior pituitaries alone from 60-120-day-old male Wistar-Imamichi rats. The pituitaries were dissociated by pipetting, and incubated for 30 min in Petri dishes containing 300 pronase units (PU) ml⁻¹ of Dispase (Godosyusei, Tokyo). After about 5 months, contact-inhibited fibroblasts were removed as a confluent cell sheet. The remaining cells consisted largely of glandular and nerve-like cells; these were incubated in 0.1% trypsin solution (in Hanks' balanced salt solution) at 4 °C.

Fig. 1 **A**, Phase contrast of the JH-S3 clonal strain derived from adult rat anterior pituitary. Long cellular projections are present which are attached to other cells, but in the proliferating stage these become spherical. The clonal cells were grown in growth medium (82.5% Ham's F10+10% newborn calf serum+5% fetal calf serum+2.5% donor horse serum). All sera were from Flow Laboratories. $\times 185$. **B**, Immunocytochemistry, using anti-S100 protein IgG, reveals sharply defined concentrations of stain in the juxtanuclear cytoplasm of clonal cells and in the long cellular projections (arrows). The cells, on coverslips, were fixed with mercuric chloride-formol (70% saturated mercuric chloride, 30% formol) for 20 min, de-mercuric chloride with 5% iodine-70% alcohol, de-iodine with 0.25% sodium thiosulphate solution, then rinsed several times with 0.02M phosphate-buffered saline (PBS) at pH 7.4. The cells were then incubated with rabbit anti-IgG fraction (7 μ g protein per ml PBS) against bovine S-100 protein at 30 °C for 1.5 h, followed by incubation with peroxidase-labelled anti-rabbit IgG (goat IgG fraction), dipped into (DAB) solution, and immersed in DAB-H₂O₂ solution to visualize the immunoreaction under microscopy. For immunocytochemistry, we prepared an anti-S100 protein antiserum in a Japanese white rabbit. The bovine S-100 antigen was supplied by Okuyama²⁵. The cross-reactivity of the antiserum with bovine and rat S-100 proteins was checked by the Ouchterlony double-diffusion test²⁸. The specificity of the antiserum was also checked by allowing it to immunoadsorb to S-100 molecules coupled to CNBr-activated Sepharose 4B (Pharmacia); immunostaining was eliminated. The eluate from the immunoabsorbent possessed immunoactivity, and was used as an anti-S-100 protein IgG fraction. $\times 185$. **C**, JH-S3 clonal cells are characterized by well developed Golgi apparatus (G), rough endoplasmic reticulum and polysomes. Interdigitated cell-to-cell contacts are usually observed between the monolayered cells. There are no secretory granules but lysosomes are frequently observed. The cells were fixed with 1% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 and post-fixed with 1% OsO₄ in Millonig's buffer at pH 7.4. $\times 2,540$. **D**, JH-S3 cells were transplanted into the subepidermis of BALB/c nude mice, fixed with mercuric chloride-formol, embedded in Paraplast, and sectioned at 2 μ m with glass knives. After treatment with de-mercuric chloride, the sections were stained immunocytochemically with the anti-S100 protein IgG fraction, then dipped into DAB-H₂O₂ solution. Strongly and weakly immunostained cells usually have long cell projections (arrows). $\times 185$.



Nerve-like cells were removed under phase contrast microscopy by using a micromanipulator equipped with a 50 μ m micropipette. After cell growth was evident, the single cell-plating feeder layer method⁶ was used to establish three strains: JH-S3, JH-S8 and JH-S12. The clonal cells have cytoplasmic projections which make contact with each other (Fig. 1A). Secretory granules are not observed, but well developed Golgi apparatus, rough endoplasmic reticulum and numerous lysosomes are visible (Fig. 1C). Desmosomes, characteristic of follicular cells, were not seen in the cultures or in the grafts of the clonal cells. The electron micrograph of JH-S3 clonal cells showed a resemblance to folliculostellate cells. Of the three clonal strains, JH-S3 reacted strongly to anti-S100 protein antiserum (Fig. 1B), while the staining intensities of the other strains were weaker. The weaker staining of the latter two strains may be due to lower production of the S-100 protein. The number of immunopositive cells increased after the cells attained confluence and round or ovoidal cells were immunonegative or only weakly stained. The JH-S3 cells frequently had long cell projections which terminated on neighbouring cells and sometimes fused with the neighbour's cytoplasm (Fig. 1C). In monolayer cells or in the grafts, there were no luminal surfaces and junctional complexes characteristic of follicular cells. This clonal strain had a stem line of 40-44 chromosomes, and the population doubling time of the culture varied from 48 to 52 h with 1×10^5 cells ml⁻¹ present at the sixth passage after establishment. When 1×10^5 JH-S3 cells were implanted into the subepidermis of nude mice, a solid tumour was formed which contained S-100 protein (Fig. 1D).

The S-100 protein is serologically similar among a wide variety of vertebrate species². The protein has been shown to

facilitate transport of γ -aminobutyric acid across nerve-cell membranes²¹ and it interacts with artificial lipid membranes to induce increased permeability to cations in physiological conditions²². It also increases activity of nuclear RNA polymerase in nuclei isolated from immature chick brains²³ and undergoes orthodromic axonal transport²⁴. Thus, the S-100 protein has an important role in nervous tissue.

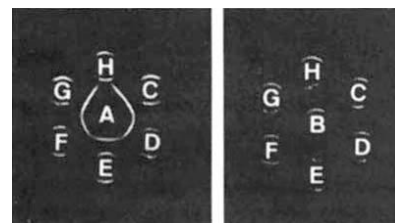


Fig. 2 Double-diffusion analysis of the S-100 protein antiserum was performed to check the formation of specific antigen-antibody complex according to Ouchterlony²⁹. The wells contained the following: A, antiserum against the purified bovine S-100 protein; B, antiserum adsorbed with purified S-100 protein (100 μ g per 0.1 ml of antiserum; Wako); C, soluble extract of rat brain; D, soluble extract of rat anterior pituitary; E, culture medium from JH-S3 cells concentrated 250-fold by ammonium sulphate precipitation; F, purified bovine S-100 protein (100 μ g ml⁻¹); G, soluble extract of JH-S3 cells; H, sera used in the growth medium. The five precipitin lines in the left-hand part of the figure were completely congruent with each other without any spurious formation; however, a precipitin line was not observed between the mixed sera and anti-S-100 protein antiserum, or when the antiserum was adsorbed with S-100 protein (right-hand part of figure).

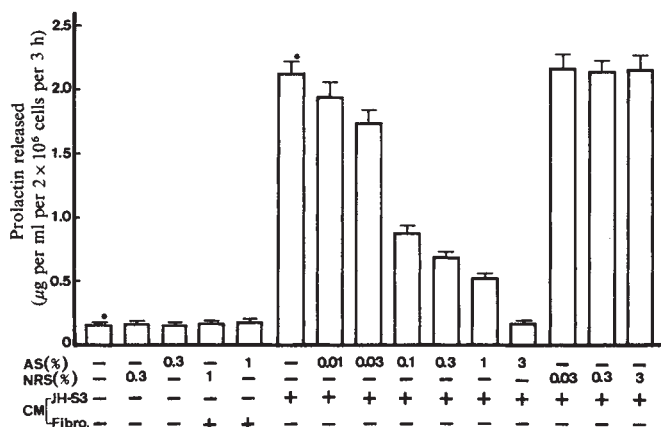


Fig. 3 Amounts of prolactin released by 1G4 clonal cells cultured in different media. Columns represent the mean (of six dishes) with the bars showing the s.e.m. A significant difference ($P < 0.001$) between prolactin levels in control culture (GM) and in 10% conditioned medium of JH-S3 clonal cells, is denoted by an asterisk. The 1G4 clonal cells, derived from Rathke's epithelium of rat fetuses, secrete only prolactin; the prolactin released into the medium was measured in duplicate by radioimmunoassay. The prolactin kit used was provided by the National Agency, NIAMDD, NIH (Bethesda, Maryland), and the results are expressed in terms of NIAMDD rat prolactin-RP-2. Growth medium (GM) contained 82.5% Ham's F10 medium + 5% fetal calf serum + 10% newborn calf serum + 2.5% donor horse serum. The conditioned medium from JH-S3 cells or fibroblasts was prepared as follows: JH-S3 cells (4×10^6 cells ml^{-1}) or fibroblasts (4×10^6 cells ml^{-1}) were cultured in GM for 3 days. Then the conditioned media (CM) were collected and filtered then both media were diluted to 10% using GM. Normal rabbit serum (NRS) was obtained from a Japanese white rabbit; preparation of the rabbit anti-S-100 antiserum (AS) is described in Fig. 1 legend. To prepare each concentration of AS (not affinity-purified) or NRS, the serum was diluted with GM. The 10% conditioned medium of JH-S3 clonal cells in GM enhanced prolactin secretion about 14 times more than that of the control medium (GM). Increasing doses of AS in the growth medium supplemented with 10% conditioned medium of JH-S3 clonal cells inhibited this prolactin secretion. However, AS did not inhibit basal prolactin secretion. NRS or 10% conditioned medium of fibroblasts did not stimulate or inhibit prolactin secretion by 1G4 clonal cells.

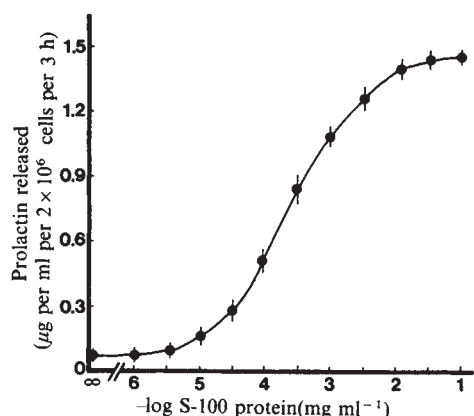


Fig. 4 The stimulatory effect of S-100 protein on prolactin secretion by 1G4 clonal cells cultured in serum-free medium (Ham's F10 medium + 0.5% bovine serum albumin) supplemented with varying doses of S-100 protein. Prolactin was assayed, as described in Fig. 3 legend, in the presence of the indicated concentrations of bovine S-100 protein (0.001 – $100 \mu\text{g ml}^{-1}$; Wako). The mean $\pm$ s.e.m. (bars) for five dishes is shown. In the control ($0 \mu\text{g S-100 protein}$), prolactin concentration in the medium was 95 ng ml^{-1} , but in the presence of $1 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$ S-100 protein, the amounts of prolactin released were 11.6 and 14.7 times higher, respectively, than the control. A concentration of $0.2 \mu\text{g S-100 protein ml}^{-1}$ yielded 50% maximum secretion.

In our experiment, conditioned medium from JH-S3 clonal cells of the anterior pituitary stimulated prolactin secretion from 1G4 prolactin-secreting clonal cells *in vitro* (Fig. 3). This prolactin secretion was inhibited by using rabbit anti-S-100 protein antiserum. Double diffusion tests revealed that: (1) the antiserum was specific for S-100 protein; (2) this protein was present in the anterior pituitary, in JH-S3 clonal cells derived from the anterior pituitary, and in the medium obtained from JH-S3 clonal cells; (3) the S-100 protein in the conditioned medium from JH-S3 clonal cells did not originate from the sera used in the growth medium (Fig. 2). Prolactin secretion was neither stimulated nor inhibited in control normal rabbit serum (NRS) or conditioned medium of fibroblasts (Fig. 3). The conditioned media from JH-S8 and JH-S12 also stimulated prolactin secretion but the effects of these were weaker than that of the JH-S3 conditioned medium. Purified bovine S-100 protein²⁵ stimulated prolactin secretion from 1G4 clonal cells cultured in serum-free medium. Figure 4 shows a dose-response curve for the amounts of secreted prolactin in relation to dose of purified S-100 protein. The conditioned medium from JH-S3 cells contained no prolactin, did not affect prolactin degradation, and did not affect prolactin recovery. These results strongly suggest that JH-S3 clonal cells synthesize and may secrete S-100 protein into the medium, and this S-100 protein stimulates prolactin secretion from 1G4 clonal cells. Calmodulin is known to participate in the release of prolactin²⁶. Our anti-S-100 protein antiserum (AS) showed no cross-reaction with calmodulin. Moreover, it did not inhibit basal prolactin secretion from 1G4 prolactin-secreting clonal cells (Fig. 3), which implies that the anti-S100 protein antiserum did not inhibit the action of calmodulin.

Immunopositive S-100 cells in the anterior pituitary appeared in 10-day-old rats, and the number of these cells gradually increased with age. In aged and castrated rats²⁷, the cells were often found surrounding the gonadotrophs. These results suggest that this protein could be related to secretion and synthesis of hormones in the rat anterior pituitary and may be involved in the so-called 'paracrine regulation'²⁸.

The successful establishment of the clonal strains reveals that this S-100 protein-producing cell is a new independent cell type of the anterior pituitary. However, the origin of this cell remains to be determined.

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Autoradiographic distribution of substance P receptors in rat central nervous system

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Among various neuropeptides present in the central nervous system (CNS), substance P, an undecapeptide¹, is of great interest as a putative pain neurotransmitter²⁻⁴. Substance P is present within numerous intrinsic neural pathways throughout the CNS^{5,6,36}. Several groups have attempted to label substance P receptors on brain membranes by ligand binding techniques⁷⁻¹²; only one study¹⁰ used native ³H-labelled substance P as the ligand and the precise anatomical distribution of substance P receptors has not yet been described. Here we report the autoradiographic localization of ³H-labelled substance P receptors in rat brain using the *in vitro* autoradiographic technique developed recently^{13,14}. ³H-substance P binds specifically to an apparently single class of sites on slide-mounted brain sections ($K_d = 0.52$ nM; $B_{max} = 21.6$ fmol per mg protein). The ligand selectivity pattern suggests that ³H-substance P binding sites are similar to those found in other assays^{11,15}. ³H-substance P receptors are highly concentrated in the external layers of the olfactory bulb, medial amygdala, dentate gyrus, superior colliculus, dorsal parabrachial nucleus and locus coeruleus, with moderate densities being found in the nucleus accumbens, striatum, periaqueductal grey and subiculum. The distribution of ³H-substance P receptors suggests that substance P is probably involved in the control of sensory processes such as pain, vision, audition and olfaction.

Slide-mounted rat brain sections were prepared as described previously¹³. Briefly, male Sprague-Dawley rats (200 g) were decapitated and their brains were rapidly immersed in isopentane at -40 °C, mounted on cryostat chucks and cut into 25-µm-thick coronal sections at -14 °C. Sections were thaw-mounted near the edge of precleaned gelatin-coated slides, dried under vacuum at 4 °C for ~2 h and stored at -70 °C for at least 48 h before use. In biochemical experiments optimal conditions were as follows. Frozen slide-mounted brain sections (A ~ 7,000–9,000 µm, according to König and Klippel¹⁶) were pre-incubated for 15 min in 50 mM Tris-HCl, 0.2 mg ml⁻¹ bovine serum albumin (BSA; Sigma), pH 7.4 at room temperature, then incubated for 30 min in 50 mM Tris-HCl, 0.2 mg ml⁻¹ BSA, 40 µg ml⁻¹ bacitracin (Sigma), 4 µg ml⁻¹ leupeptin (Sigma), 2 µg ml⁻¹ chymostatin (Sigma), 3 mM MnCl₂, pH 7.4 at room temperature with various concentrations (for saturation experiments) or 1.5 nM of ³H-substance P (28.5 Ci mmol⁻¹; NEN) and various substance P analogues or other drugs as indicated. At the end of the incubation the slides were placed in racks and transferred sequentially through four rinses (30 s each) of 50 mM Tris-HCl plus 0.2 mg ml⁻¹ BSA, pH 7.0 at 4 °C. Binding of ³H-substance P to the tissue slice was quantitated by scraping off dried sections, mixing them in 1 ml Protosol, storing them overnight and then assaying in 10 ml of Econofluor scintillation cocktail (NEN). Specific binding was calculated as the difference in radioactivity bound in the presence and absence of 1 µM substance P (Peninsula). Incubated slides were prepared for microscopic visualization as follows. At the end of the rinsing period, slides were rapidly dried under a stream of cold air and then juxtaposed tightly against tritium-sensitive film (Ultrofilm; LKB) and stored at room temperature for 4 weeks. After this exposure, films were processed in Kodak D19 at 22 °C for 4 min, then fixed for 5 min. Analysis of the

Table 1 Inhibition of specific ³H-substance P binding to rat brain sections by related peptides

Peptide	Rat brain binding IC ₅₀ (nM)	Relative potency	Mouse mesencephalic cell culture Relative potency*
Substance P	2.2	100	100
Substance P(3–11)	70	3.1	16.8
Substance P(5–11)	870	0.3	3.3
Substance P(7–11)	9,800	0.02	0.01
Substance P-COOH [D-Pro ² ,D-Trp ^{7,9}]	>50,000	0.01	Inactive
substance P	19,500	0.01	NT
Physalaemin	14	16	18
Eledoisin	260	0.8	0.9

IC₅₀ is the concentration displacing 50% of binding of 0.66 nM ³H-substance P. Values represent the mean of two experiments, each performed in triplicate. Incubation was as described in the text, in the presence of peptidase inhibitors such as bacitracin (40 µg ml⁻¹), leupeptin (4 µg ml⁻¹) and chymostatin (2 µg ml⁻¹). All substance P fragments and analogues were obtained from Peninsula. Other peptides that did not displace ³H-substance P at 1 µM were bombesin, secretin, ranatensin, glucagon, somatostatin, α-melanocyte-stimulating hormone, vasoactive intestinal peptide, β-endorphin, dynorphin, [Met]enkephalin and [Leu]enkephalin. In a typical experiment, the mean (±s.e.m.) total binding was 649 ± 9.6 c.p.m. per section and the nonspecific binding (in the presence of 1.0 µM substance P) was 122 ± 4.2 c.p.m. per section (n = 7). NT, Not tested.

*Data obtained from ref. 11.

film was performed as described previously, with underlying tissue sections counter-stained to facilitate the identification of brain microregions¹³.

Figure 1 shows a saturation curve and Scatchard analysis of ³H-substance P binding which revealed an apparently saturable single class of binding sites with a dissociation constant, K_d , = 0.52 nM and a number of sites, B_{max} , = 21.6 fmol per mg protein. These values are similar to those previously described for ³H-substance P binding using membrane-binding techniques (K_d values varied from 0.38 to 1.0 nM)^{10,17}.

Table 1 shows the relative potency of various substance P fragments and analogues in displacing ³H-substance P binding. Substance P and physalaemin, a structurally related peptide isolated from amphibian skin¹⁸, are highly potent in binding competition experiments against ³H-substance P, and the C-terminal fragments of seven or more amino acids possess appreciable activity (Table 1). The critical importance of the C-terminal amido group is illustrated by the weakness of substance P-COOH in displacing ³H-substance P binding (Table 1). These data are in agreement with results obtained in various assays^{10–12,15,17,19,20}, especially for mouse mesencephalic cells in primary culture ($r = 0.89$; $P < 0.005$)¹¹.

On the basis of results obtained in various bioassays, it has been suggested that substance P interacts with more than one class of receptors (for reviews, see refs 15, 19). Lee *et al.*²⁰ have suggested the existence of substance P-P (for physalaemin) receptor where substance P, physalaemin, eledoisin and kassinin are almost equipotent and substance P-E (for eledoisin) receptor, for which the rank order of potency is kassinin > eledoisin > substance P = physalaemin. Our results suggest that we are probably examining the substance P-P receptor subtype as eledoisin is definitively not more potent than substance P (Table 1). Thus far, using either ³H-substance P, ¹²⁵I-substance P (in preparation) or ¹²⁵I-physalaemin (in preparation), we have not found any evidence for the existence of substance P-E receptors in rat brain. Finally, [D-Pro²,D-Trp^{7,9}]-substance P, a putative substance P antagonist²¹, is a weak displacer of ³H-substance P binding (Table 1) and might explain the relatively low potency of this antagonist in various bioassays.

The autoradiographic visualization of ³H-substance P binding sites shows that substance P receptors are widely distributed in rat brain (Fig. 2). The highest densities of ³H-substance P bind-

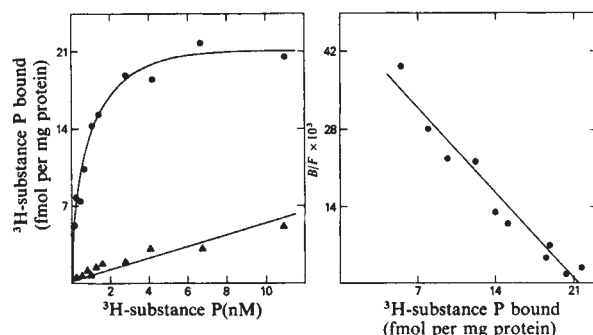


Fig. 1 Specific (●) and nonspecific (▲) binding of ^3H -substance P to rat striatal sections as a function of the concentration of ^3H -substance P (left) and as a Scatchard analysis (right). Slide-mounted rat striatal sections were preincubated for 15 min in 50 mM Tris-HCl, 0.2 mg ml $^{-1}$ BSA, pH 7.4 at room temperature and then incubated in 50 mM Tris-HCl, 1.2 mg ml $^{-1}$ BSA, 40 mg ml $^{-1}$ bacitracin, 4 mg ml $^{-1}$ leupeptin, 2 mg ml $^{-1}$ chymostatin, 3 mM MnCl $_2$, pH 7.4 at room temperature. In preliminary experiments, we observed that specific ^3H -substance P binding reaches equilibrium between 20 and 30 min, therefore we performed all subsequent incubations for 30 min. Moreover, HPLC analysis indicated that after a 15 min incubation in our conditions, 91% of added ^3H -substance P was still present in its native form. Also, immediately after receipt of one batch of ^3H -substance P (NEN), 10- μl aliquots were transferred to small plastic tubes, flushed with argon and then stored under liquid nitrogen until used. Nonspecific binding in the presence of 1.0 μM substance P has been subtracted from all experimental points. Values are the mean of three or more determinations and varied by less than 20%.

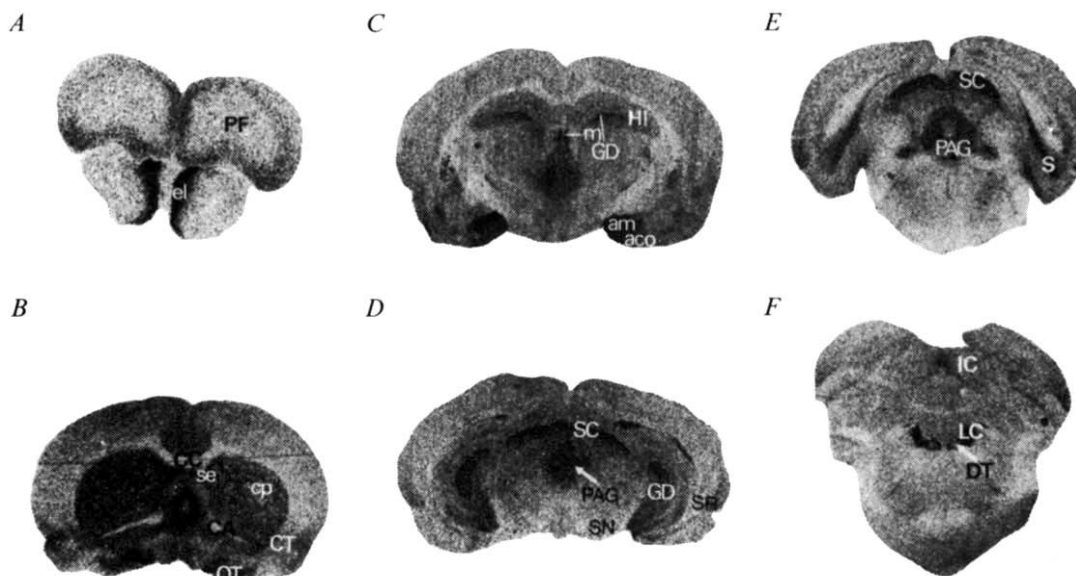
ing sites are present in the external layers of the olfactory bulb (Fig. 2A), olfactory tubercle, septal nuclei (Fig. 2B), preoptic nucleus, medial nucleus of amygdala (Fig. 2C), medial habenula (Fig. 2C), dentate gyrus (Figs 2C, D and 3A), superior colliculus (Figs 2D, E and 3B), locus coeruleus (Figs 2F, 3C) and dorsal parabrachial nucleus. More specifically, the highest densities of ^3H -substance P binding sites in the dentate gyrus are found in the granular layer with lower densities in adjacent structures such as the hilus fascia dentata and the stratum lacunosum moleculare (Fig. 3A). In the superior colliculus, the highest concentration is observed in the stratum griseum superficiale (Fig. 3B). In the brain stem, the locus coeruleus is very rich in ^3H -substance P receptors compared with the dorsal tegmental nucleus (Fig. 3C). In other areas, moderate levels of ^3H -substance P receptors are found in the nucleus accumbens, striatum (Fig. 2B), pyriform cortex (Figs 2B, C), central and

cortical nuclei of amygdala, nucleus gelatinosus (Fig. 2C), claustrum (Fig. 2C), hippocampus (Figs 2C, D), paraventricular nucleus of the hypothalamus, periaqueductal grey (Fig. 2E), subiculum (Fig. 2E), entorhinal cortex (Fig. 2E), inferior colliculus (Fig. 2F) and dorsal horn of the spinal cord. Low to moderate concentrations of sites are observed in the globus pallidus, most cortical and hypothalamic areas and dorsal raphe (Fig. 2F). Low densities are present in most thalamic areas (Fig. 2C), the cerebellum and, surprisingly, the substantia nigra (Fig. 2D). White matter and controls which had been treated with 1 μM substance P showed negligible levels of binding. Overall, the ^3H -substance P binding site distribution is very similar to those found for ^{125}I -substance P 22 and ^{125}I -physalaemin (in preparation).

The distribution of ^3H -substance P receptors does not correlate perfectly with that of endogenous substance P. Similar discrepancies have been observed for many other neuropeptides 23,24 as well as for various neurotransmitters 24,25 . In the case of substance P the absence of substance P binding sites in the substantia nigra is particularly striking as high levels of substance P-like immunoreactivity have been reported to exist in this region 6 and behavioural and biochemical effects of substance P after intranigral injections have been documented 26 . However, very negligible electrophysiological effects of substance P on substantia nigra pars compacta dopaminergic cell bodies have been reported 27 . Our results may suggest that a different class of substance P receptors is present in the substantia nigra and only appropriate incubation conditions will reveal it.

The high concentrations of ^3H -substance P receptors in many brain areas associated with sensory input suggest that substance P might be an important neurotransmitter and/or neuromodulator in the integration of sensory information. For example, the high density of receptors found in the external layer of the olfactory bulb and in the olfactory tubercle could indicate a role for substance P in olfaction. Similarly, substance P receptors in the superior and inferior colliculi might be involved in visual and auditory information processing, respectively. Already, substance P-like peptides have been found in the eye 28 and it is known that the superior colliculus receives direct visual input from the retina, as well as from the visual cortex 29 . Also, because of the probable involvement of substance P in pain mechanisms 2 , it is possible that certain substance P receptors in the inferior and superior colliculi could be associated with pain-activated fibres from the spinal cord that end in the colliculi (spinotectal tract) 30 . The moderate to high concentrations of

Fig. 2 Photomicrographs of ^3H -substance P receptor distribution in rat brain. Brain sections were processed as described in the text. A, A forebrain section, A 12,130 μm ; B, a striatal section, A ~7,470 μm ; C, a midbrain section, A 3,990 μm ; D, a midbrain section, A ~1,610 μm ; E, a midbrain section, A ~620 μm ; and F, a brain-stem section. CA, commissura anterior; CC, corpus callosum; CT, claustrum; DT, dorsal tegmental nucleus; GD, dentate gyrus; HI, hippocampus; IC, inferior colliculus; LC, locus coeruleus; PAG, Periaqueductal grey substance; OT, olfactory tubercle; PF, polus frontalis of cortex; S, subiculum; SC, superior colliculus; SN, substantia nigra; SR, rhinal sulcus; aco, cortical nucleus of amygdala; am, medial nucleus of amygdala; cp, caudate putamen; el, external layers of olfactory bulb; m, medial habenula; and se, nucleus septi lateralis.



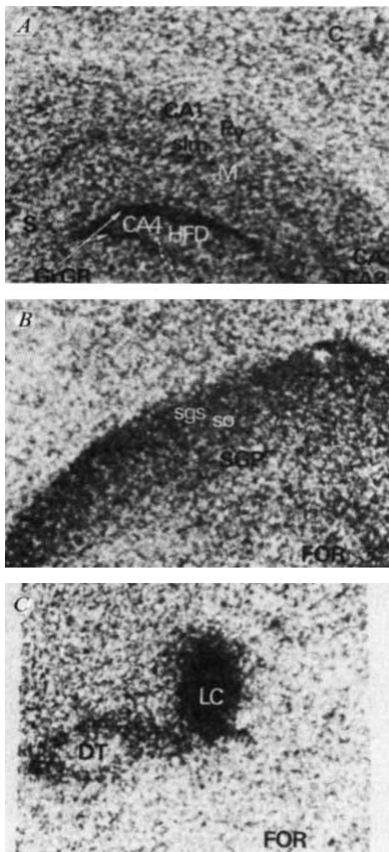


Fig. 3 Photomicrographs of ^3H -substance P receptor distribution in hippocampus (A), superior colliculus (B) and locus coeruleus (C) of the rat. Brain sections were processed as described in the text. C, cortex; CA1, CA2, CA3, CA4, cornu ammonis area 1, 2, 3 and 4, respectively; DT, dorsal tegmental nucleus; FOR, formatia reticularis; GrGR, granular layer of dentate gyrus; HFD, hilus fascia dentata; LC, locus coeruleus; M, stratum moleculare of the dentate gyrus; Py, pyramidal cell layer of hippocampus; S, subiculum; SGP, stratum griseum profundum colliculi superioris; sgs, stratum griseum superficiale colliculi superioris; SO, stratum opticum colliculi superioris; and slm, stratum lacunosum moleculare.

^3H -substance P receptors in the periaqueductal grey substance and dorsal horn of the spinal cord also support the possible role of substance P in pain information transfers.

Another system where substance P might be an important modulator is in the nigrostriatal dopaminergic pathway. It is known that administration of substance P in the substantia nigra stimulates the release of ^3H -dopamine³¹ and increases homovanillic acid and 3,4-dihydroxyphenylacetic acid levels in the ipsilateral striatum³². This increase in dopaminergic activity correlates with an increase in rotational behaviour³³. The moderate to high concentrations of ^3H -substance P receptors in the striatum support the possible involvement of substance P in the activity of the nigrostriatal dopaminergic pathway. It would be of interest to study substance P receptors in diseases related to this dopaminergic system such as parkinsonism and Huntington's disease, especially as substance P brain levels are dramatically decreased in the latter disease^{34,35}.

The autoradiographic visualization of ^3H -substance P receptors suggests that substance P could be an important modulator of sensory input, especially as moderate to high densities of receptors have been found in the olfactory bulb and olfactory tubercle (olfaction), in the superior and inferior colliculi (vision, audition and pain) and in the periaqueductal grey substance (pain) of brain.

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Octopamine—a neurohormone with presynaptic activity-dependent effects at crayfish neuromuscular junctions

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Octopamine, the phenol analogue of noradrenaline, is a neurosecretory product found in many vertebrate and invertebrate species. In the American lobster, octopamine produces an increase in muscular tension during activation of the motor nerve and may induce spontaneous contractions at concentrations of 10^{-7} M (refs 1, 2). In the lobster, postsynaptic mechanisms, including a change in Ca^{2+} conductance of the muscle membrane, are thought to be responsible for potentiation of contraction². In contrast, studies on insects have implicated both pre- and postsynaptic effects: for example, O'Shea and Evans³ reported that neuromuscular transmission in the locust leg extensor muscle is modulated by octopamine released from a specific neurosecretory neurone which acts on high-affinity octopamine receptors located both on the muscle and on excitatory nerve terminals. The presynaptic receptors mediate an increase in frequency of spontaneous miniature postsynaptic potentials recorded in the muscle⁴. In view of the apparent discrepancy between insect and crustacean results, we have re-examined the effects of octopamine on neuromuscular transmission in a crustacean muscle and report here that enhanced postsynaptic potentials produced by very low levels of octopamine (10^{-10} – 10^{-7} M) are largely attributable to a presynaptic effect which increases quantal release of transmitter. Also, this effect is more pronounced and longer lasting when octopamine is applied to active neuromuscular preparations. This system provides a model for selective consolidation of active synapses by neurohormonal mechanisms. Such an effect could be of general significance in the nervous system, as it would provide a mechanism for selective neurohormonal regulation and strengthening of pathways used during specific activities.

The preparation used in this study was the 'opener' muscle in the claw of a freshwater crayfish (*Procambarus clarkii*). This preparation provides synapses that are physiologically similar to many central synapses^{5,6}. The animals (6–10 cm long) were maintained at 16 °C before use.

The ventral surface of the opener muscle was exposed in the propodite and set up for perfusion with buffered van Harreveld's solution (205.3 mM NaCl, 5.36 mM KCl, 13.5 mM CaCl₂, 2.46 mM MgCl₂, buffered to pH 7.4 with 10 mM HEPES). Octopamine solutions of 10^{-10} – 10^{-7} M were freshly prepared by adding D,L-octopamine (Sigma) to the standard solution. Experiments were performed at 15 °C. The single excitatory axon to the opener muscle was stimulated and excitatory postsynaptic potentials (e.p.s.ps) were recorded from individual muscle fibres with intracellular microelectrodes while tension of opener muscle contractions was measured semi-isometrically with a force displacement transducer hooked to the dactyl of the claw; e.p.s.ps were elicited by test stimuli (10-s trains at 15 Hz) and averaged on a Nicolet 1072 Fabri-tek signal averager to obtain average amplitude. Postsynaptic effects of octopamine on membrane resistance were monitored by measuring input resistance (R_{in}) of individual muscle fibres penetrated with two electrodes: one for injection of current, the other for recording membrane voltage response. Presynaptic effects were measured by using external microelectrodes placed so as to record maximum amplitude of externally recorded synaptic currents (e.r.s.cs). These electrodes were filled with crayfish saline and had tips 20–30 μ m in diameter. Quantal content estimates were made from a series of 100 to 200 trials during stimulation at 2 Hz by using two methods: (1) number of failures; and (2) direct counting of quanta released, as described by Wernig⁷ and Zucker⁸. At these crayfish synapses, in which several failures were recorded, the two methods gave very similar estimates of quantal content. The above measurements allowed assessment of the relative contribution of pre- and postsynaptic effects to changes in e.p.s.p. amplitude.

At concentrations $\geq 10^{-10}$ M, octopamine increased the amplitude of e.p.s.ps, and enhanced total membrane depolarization during a train of stimuli (Fig. 1); muscle tension increased simultaneously. The time course of these two effects was similar. The enhancement of e.p.s.p. amplitude appeared to be dose-dependent within the range 10^{-10} – 10^{-8} M octopamine, with up to 150% increases observed for 10^{-8} M octopamine (Fig. 2A). In some preparations, effects were seen at concentrations of 10^{-11} M octopamine, and it is likely that a threshold for the octopamine effect lies between 10^{-10} and 10^{-11} M in most preparations.

We found that the magnitude and persistence of the effect of octopamine on the e.p.s.ps were very sensitive to the amount of activity occurring in the motor neurone. Figure 2B shows a typical result. When 10-s trains of stimuli at 15 Hz were delivered every 2 min, e.p.s.p. amplitude increased to 85.6% above normal over a 1-h period, returning to 40.9% above normal after a 1-h wash in normal saline. In contrast, when trains of stimuli were delivered only once every 20 min to a similar preparation, there was a <40% increase in e.p.s.p. amplitude—about half that seen in more active preparations. A striking difference in persistence of the octopamine effect in standard saline was also seen. Less active preparations recovered completely within 1 h, while more active preparations retained an enhanced e.p.s.p. for much longer than 1 h.

As maintained activity by itself can lead in some conditions to enhanced e.p.s.p. amplitude through a process of long-term facilitation^{9–11}, we checked the effects of the standard stimulation regime (one train every 2 min) on e.p.s.p. amplitude in standard solution containing no octopamine. Typically, no changes were seen over 2.5–3 h (refs 9, 11). Clearly, octopamine acted synergistically with activity to enhance e.p.s.p. amplitude.

To determine whether the effects of octopamine on e.p.s.p. amplitude are postsynaptic in origin, as claimed for lobsters², or partly presynaptic, input resistance of muscle fibres was measured during genesis and subsidence of octopamine effects.

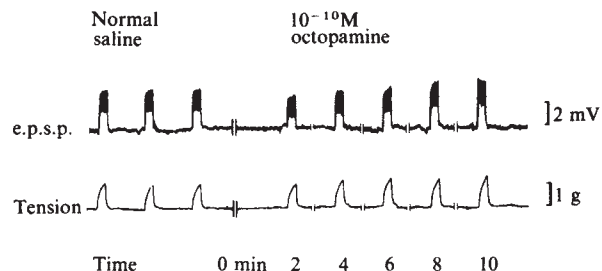


Fig. 1 Effects of 10^{-10} M octopamine on e.p.s.ps and muscle tension during a standard stimulus train (15 Hz for 10 s, delivered once every 2 min). Amplitude of e.p.s.p. and total membrane depolarization in the muscle fibre, increased slowly with time after octopamine application. A corresponding gradual increase in muscle tension also was apparent.

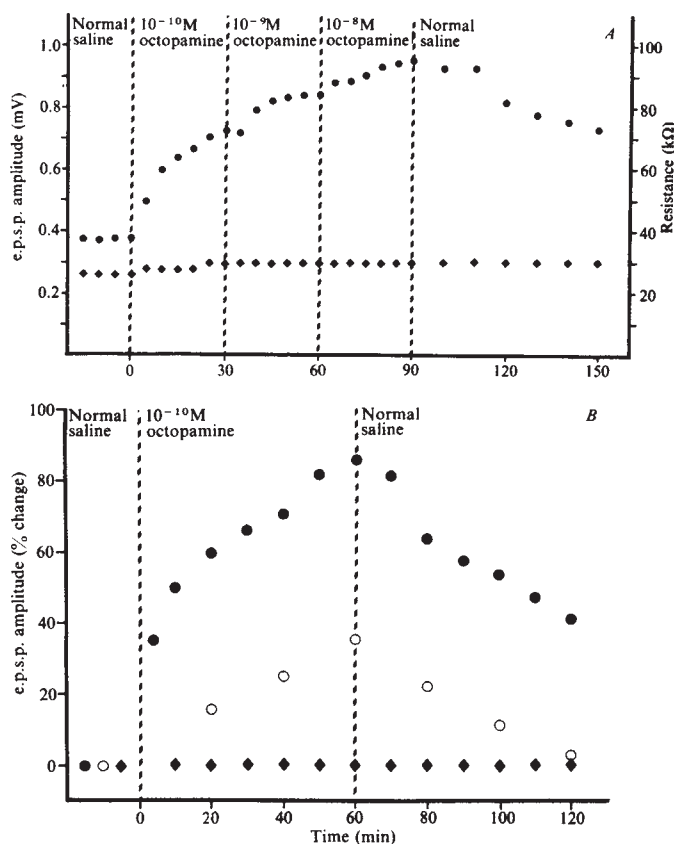


Fig. 2 A, Simultaneous measurement of e.p.s.p. amplitude (circles) and input resistance (diamonds) in a representative single muscle fibre, for several concentrations of octopamine, to show the dose dependence of the effect. An insignificant increase in the input resistance was recorded, while the e.p.s.p. increased progressively in the presence of octopamine. B, Activity-dependent enhancement of e.p.s.ps by octopamine. Open circles, percentage changes in e.p.s.p. amplitude with minimal stimulation (one train every 20 min). Solid circles, percentage changes in e.p.s.p. amplitude with more frequent stimulation (one train every 2 min). Diamonds, percentage changes in input resistance with one train delivered every 20 min.

In separate experiments, measurements of quantal content of transmission at individual synaptic sites were made with extracellular microelectrodes¹². Input resistance (which reflects specific membrane resistance) changed very little during octopamine treatment (Fig. 2A, B). In some fibres, a small increase of less than 15% was observed (Fig. 2A) and in others there was none (Fig. 2B). In preparations showing a small increase in input resistance, the change was not large enough to account for increased e.p.s.p. amplitude (Fig. 2A). Measurements of quantal content were performed on preparations

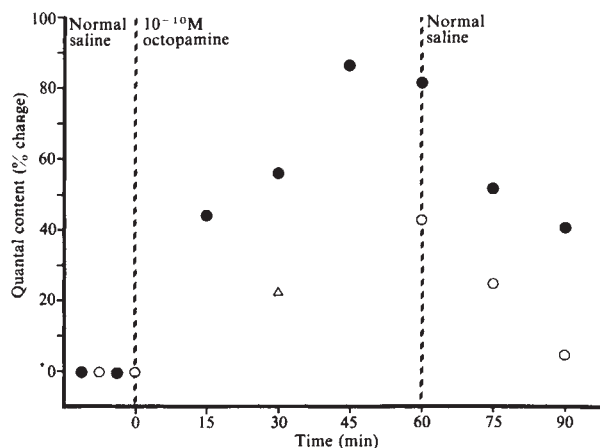


Fig. 3 Presynaptic effects of octopamine at two different activity levels. Percentage changes in quantal content were followed at representative synaptic sites during standard stimulation (one train every 2 min, solid circles) and less frequent stimulation (no background stimulation: open circles, individual determinations; open triangle, average of three determinations made between 15 and 45 min). All estimates shown were based on the proportion of failures, and were made at a stimulation rate of 2 Hz.

bathed in 10^{-10} M octopamine for 1 h and subjected to test stimuli every 2 min. Typically, there was a substantial increase in quantal content, which recovered slowly during normal saline wash (Fig. 3). Both the magnitude and the persistence of the change in quantal content were activity-dependent, as in the e.p.s.p. measurements. Furthermore, the percentage changes in quantal content were very similar to those of the e.p.s.p. With standard stimulation, quantal content increased 70–85% during 1 h exposure to octopamine, returning to 40–45% above normal after the 1 h washout. With minimal stimulation, quantal content increased only 25–40% in 10^{-10} M octopamine, and returned to normal within 1 h during washout (Fig. 3). Very good agreement with e.p.s.p. measurements was obtained; changes in quantal content can account for most if not all of the enhancement of e.p.s.ps.

No obvious changes in the time course of individual quantal events were observed. The mean time constant of decay of the e.r.s.c., measured for a sample of 25 quantal events recorded in normal Van Harreveld's solution, was 2.94 ± 0.17 ms (s.e.m.). After 30 min in 10^{-10} M octopamine, the corresponding value was 3.06 ± 0.19 ms; after 60 min, 3.02 ± 0.16 ms. The lack of significant change in the time constant of decay of the quantal synaptic current indicates that octopamine does not markedly influence the kinetics of the postsynaptic channels that generate the e.p.s.p.

The present results provide strong evidence for a presynaptic effect of octopamine at the crayfish neuromuscular synapse, supporting an earlier suggestion¹³. Presynaptic octopamine receptors are believed to occur at insect neuromuscular junctions^{3,4} and our evidence suggests that they may also occur in the crayfish. The reported lack of presynaptic effect of octopamine in lobsters⁵ may reflect a species difference, but in view of the results in crayfish, measurements of quantal release should be made at lobster synapses. It is possible that an effect of octopamine on muscle fibre metabolism may also occur, leading to increased tension; this was not studied in the present experiments.

Of particular interest is the activity dependence of the octopamine effects. Both the magnitude and duration of the octopamine effects were augmented by higher levels of background activity. Although the cellular mechanism of this effect must remain speculative pending further investigations, the functional consequences may be significant. The effect provides an example of neurohormonal modulation which selectively enhances more active synapses. If such effects also occur in the central nervous system, in response either to octopamine or to

other neurohormones, pathways that are active at the time that the neurohormone is released into the circulation would be selectively strengthened. Behavioural actions could be facilitated. Depending on the level of activity and persistence of changes initiated by the neurohormone, enhancement of a neural circuit could be either transient or permanent, and could be of particular importance during development.

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Rapid transport of foreign particles microinjected into crab axons

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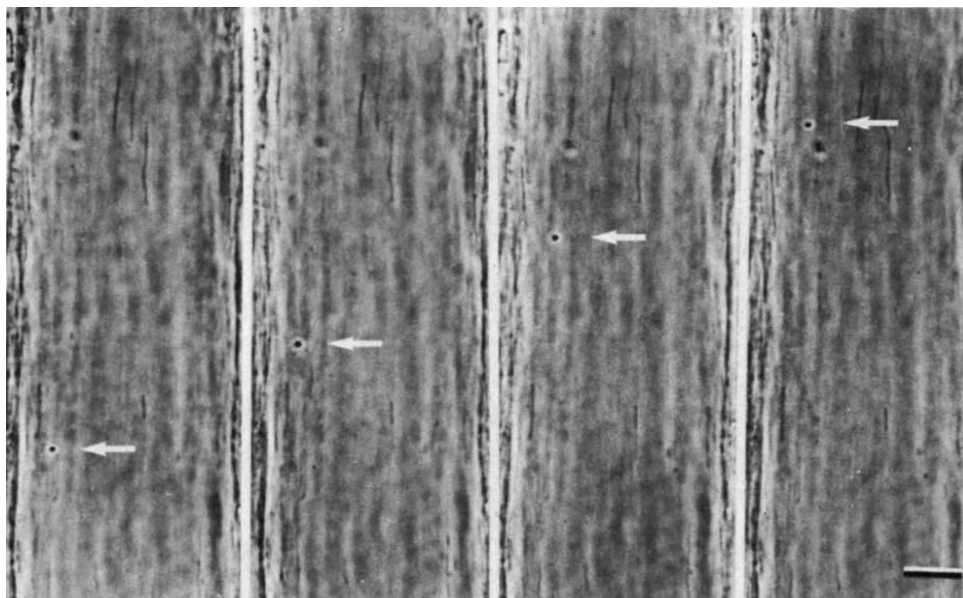
The rapid transport of optically detectable organelles in axons has been well documented, although its molecular mechanism remains unknown^{1–3}. Here we report that synthetic particles microinjected into the giant axons of the shore crab, *Carcinus maenas*, are also transported, moving as though they were endogenous organelles. Polystyrene beads, polyacrolein beads, paraffin droplets and glass fragments, of sizes up to 0.5 μ m in diameter, have been tested. Many of these foreign particles move rapidly and for long distances along the axon in the anterograde direction, travelling in a saltatory fashion, within a well defined velocity range. In many respects the movements are indistinguishable from those of anterogradely moving endogenous organelles seen by phase-contrast in these axons. Our results indicate that there is a transport system in axons capable of carrying almost any particle of suitable physical properties in an anterograde direction.

Single giant axons were dissected from the walking legs of the shore crab *C. maenas* and mounted for observation in Petri dishes with coverslip inserts in a buffer resembling their internal ionic composition (400 mM K glutamate, 150 mM glycine, 10 mM EGTA, 20 mM HEPES pH 7.2, 5 mM $MgCl_2$), as previously described⁴. Before use, the glass coverslips were chemically modified to give free covalently bonded aldehyde groups on their surface, making them highly adhesive⁵. Axoplasmic transport was observed in these axons using a $\times 100$ phase-contrast objective on an inverted microscope (Zeiss IM35) mounted on an air-isolation vibration-free table (Backer-Loring, Peabody, Massachusetts). Organelles were seen to move both retrogradely and anterogradely for at least 2 h in these conditions.

Needles for microinjection were made from glass capillaries with an internal filament (Clark Electro Medical Instruments, Pangbourne) and were pulled according to the technique of Graessman *et al.*⁶ using a microelectrode puller to pre-pull the capillaries. Needles with a final tip aperture of 0.8–1.0 μ m were back-filled by capillary action with the suspension of particles to be injected. The filled needles were attached to a 50-ml syringe by Teflon tubing to provide the air pressure for injection and held in a Leitz micromanipulator.

Polystyrene beads of 0.37 or 0.5 μ m diameter (Agar Aids, Bishops Cleeve) were suspended in the artificial internal buffer for microinjection into the axon. To avoid disrupting the

Fig. 1 From left to right, a sequence of photographs taken at 25-s intervals showing a 0.5- μm diameter polystyrene bead moving anterogradely in a crab axon. Scale bar, 10 μm .



axon excessively, as small a volume as possible was injected, the number of beads introduced into the axon varying from one to several dozen. Within 30–60 s of entry into the axon, the beads showed an oscillatory motion, 1–2 μm in amplitude; this was distinct from brownian motion and directed along the long axis of the axon. The delay before beads began to move was very variable: some started almost immediately whilst others took many minutes or did not move at all during the experiment. Once moving, the beads travelled uniformly, always in the anterograde direction, in an almost identical fashion to endogenous organelles moving in that direction (Figs 1, 2). The chief difference between the movement of injected beads and endogenous organelles was that beads tended to spend longer periods stationary between saltations. The frequency distributions of instantaneous velocities of endogenous organelles and polystyrene beads (Fig. 3) are very similar. Permeabilization of axons by high-voltage discharge⁴ showed the movement of polystyrene beads, like that of endogenous organelles, to be ATP dependent.

The movements of beads mimicked those of organelles in one other pattern of motion. Occasionally, organelles and beads were seen to undergo a very rapid recoil in the direction opposite to their predominant movement⁷; this reversal of motion sometimes occurred several times before the organelle made any net advancement. The velocity of recoil was not measured but was faster than other movements seen in the axon and gave the impression of being elastic in nature. Because it occurred more frequently in axons that had become less ordered, it may reflect a breakdown of the highly structured arrangement of axoplasm. It is also interesting that both retrogradely and anterogradely moving organelles show such recoils.

Little difference was seen in the frequency distribution of instantaneous velocities of polystyrene beads of 0.37 μm or 0.5 μm diameter (Fig. 3), but when beads of 0.8 μm diameter were injected they were able to make only small, 1–2 μm , displacements. These larger beads could, however, be made to move by reducing the osmotic strength of the bathing medium, which causes a slight swelling of the axon. Further types of polystyrene beads were also injected into axons. Ordinary polystyrene beads, as used above, possess a small negative charge but highly charged polystyrene beads, carrying either negatively charged carboxylate or positively charged aliphatic amine residues on the surface, are commercially available (Polyscience Inc., Warrington, Pennsylvania). When such beads with a negative surface charge (diameter 0.45 μm) were injected they were

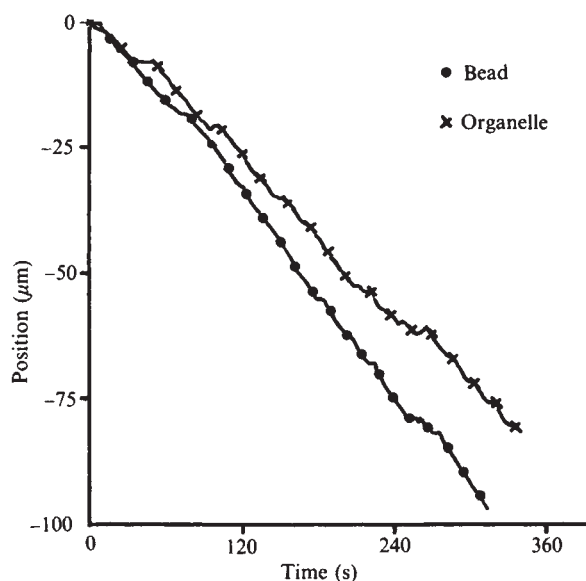


Fig. 2 A plot of position versus time for a typical anterogradely moving organelle ($\times$) and for a 0.37- μm diameter polystyrene bead ($\bullet$). The data were obtained by measuring the time taken for a particle to pass 0.9- μm divisions on an eyepiece graticule. Markers were plotted at every fifth measurement.

again seen to move. Those with a positive charge showed very little movement, after 10 or more minutes, exhibiting small oscillations, or short and rare displacements. Similarly, negatively charged beads, preincubated with 1 mg ml⁻¹ poly-L-lysine (type II, Sigma), showed no tendency to move.

To define the requirements of transport further, other types of particle were injected. Paraffin droplets, prepared by sonicating 25% (v/v) paraffin oil in internal buffer with 5 mg ml⁻¹ bovine serum albumin (BSA) to act as a surfactant⁸ showed movement when injected. Hydrophilic polyacrolein beads, made according to the method of Margel *et al.*⁹, were also seen to move. These beads were suspended in the internal buffer for some time before injection so it is likely that many if not all of the free aldehyde groups would have reacted with the amino acids in the solution. Further, beads incubated overnight in 5 mg ml⁻¹ BSA were still transported when injected into

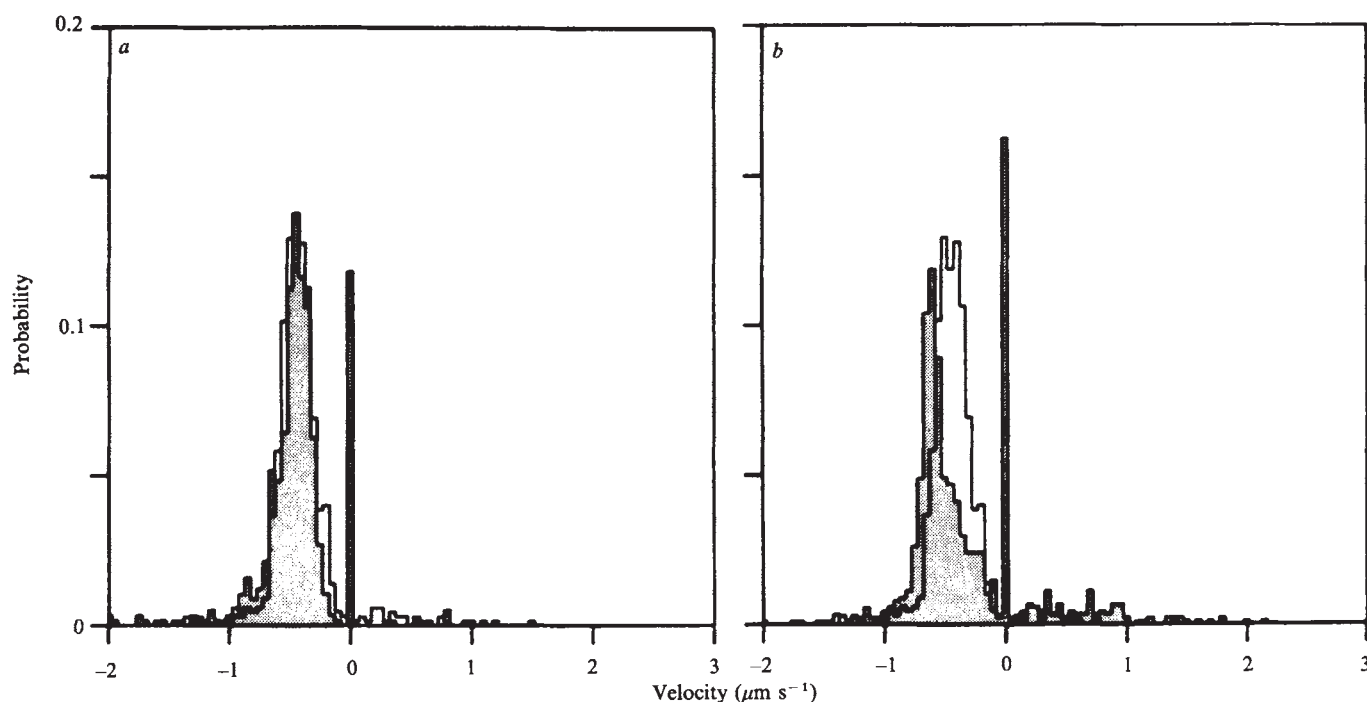


Fig. 3 A comparison of instantaneous velocities calculated from the data collected by the technique described in Fig. 2. In both graphs, clear bars represent endogenous organelles and shaded bars the appropriately sized beads. *a*, 0.37- μm diameter beads; *b*, 0.5 μm diameter. This figure represents the accumulated data of 649 velocities from 9 endogenous organelles: 558 velocities from 6 polystyrene beads of 0.37 μm diameter; and 874 velocities from 7 polystyrene beads of 0.5 μm .

Table 1 Foreign particles injected into axons, scored for their ability to move in saltatory fashion for long distances along an axon following injection

Particle	Movement	Direction
Polystyrene	+	Anterograde
Polystyrene (COO^-)	+	Anterograde
Polystyrene (NH_4^+)	—	—
Polystyrene/polylysine	—	—
Polyacrolein	+	Anterograde
Polyacrolein/BSA	+	Anterograde
Paraffin/BSA	+	Anterograde
Glass	—	—

axons. Finely ground glass was filtered through a 0.6- μm Millipore filter to give a population of suitably sized although irregularly shaped particles. When injected into axons they showed no tendency to move, while endogenous transport of optically detectable organelles continued unabated. Glass particles have a negative surface charge. Taken together (Table 1), our results suggest that there are few restrictions on the chemical nature of particles that will move in axons. Negative charges appear to be essential, but both hydrophobic and some hydrophilic particles (except glass) are able to move.

We can distinguish three mechanisms by which these foreign particles might move. First, they might be carried by an indiscriminate transport mechanism such as a localized fluid stream—capable of moving any physically suitable object in their locality¹⁰. The failure of some types of particles to move may, in this case, be due to their preferential association with stationary components of the axoplasm. Second, the particles could move through the adherence of suitable proteins from the axoplasm onto their surface. Polystyrene beads, in particular, readily adsorb proteins from solution, including both actin and myosin^{11,12}. (We should remember, however, that particles whose surface was already coated with bovine serum albumin nevertheless moved.) Third, injected particles could move piggy-back fashion, through an association with another

component of the axon that is, itself, undergoing transport, such as a transport filament, or a vesicle. No interaction with organelles large enough to be visible in phase contrast was seen, but there does exist a population of much smaller membrane-bounded organelles, moving anterogradely, which are beyond the detection of our equipment⁷.

These three possible mechanisms overlap to some extent, because a steady flow of submicroscopic particles confined to a particular channel in the axoplasm would have many of the properties of a localized fluid stream, and an association of a particle with a small vesicle or filament undergoing transport is not very different from the surface adsorption of a protein complex capable of conferring movement.

Any of the above mechanisms could, in principle, operate in the normal movement of optically detectable organelles. However, endogenous organelles travel in either the retrograde or the anterograde direction, persisting in their direction of movement for long distances, whereas all of the injected particles travelled exclusively in the anterograde direction. This seems to imply that retrograde transport involves a different system—possibly one with a more stringent requirement for the type of particle carried. We may be able to investigate this further by injecting particles coated in more specific ways.

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Variation in regulation of steroid sulphatase locus in mammals

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Inactivation (lyonization) of one of the two copies of X-linked genes occurs in female mammals, thereby reducing the number of active copies to that of the male. It has been suggested that genes subject to lyonization would be expected to be preserved as a linkage group during mammalian evolution¹. A short region of the human X chromosome containing several genes, including that necessary for the expression of steroid sulphatase (STS), is exceptional in that it apparently escapes X-inactivation². As it is not apparent why the linkage of genes not subject to X-inactivation should be conserved, we have examined the expression of the STS gene in mice (it has been shown recently that this gene is X-linked³). Enzyme levels were determined in normal males and females and in the progeny of crosses in which the sex reversing factor, *Sxr*, was segregating to produce XX males⁴. We report here that in contrast to the situation in humans, the STS gene in mice is subject to the normal pattern of X-inactivation.

Three types of material were used in the determination of STS activity. Skin fibroblasts of 23 embryos (18 days post coitum) and heart fibroblasts of 12 adults (8 weeks) from an inbred mouse strain (BALB/c) were cultured and assayed in duplicate to compare the male and female levels. In addition, liver extracts from 25 offspring of matings producing XX *Sxr* males were assayed for STS activity. The progeny of such matings were chosen for study because XX males provide an internal control for evaluating the possible influences of hormonal differences between the sexes on STS activity. XY males carrying *Sxr* were mated to normal females (all mice of mixed C3H/HeH and 101/H background), giving rise to offspring of four types, produced in equal proportions: XY *Sxr* males, XY non-*Sxr* males, XX *Sxr* males and XX females. The XX *Sxr* males are sterile, though apparently physiologically normal, and have very small testes due to the absence of germ cells. They were distinguished on this basis from the XY classes⁴. XY *Sxr* males were distinguished from XY males by their abnormally high level of XY separation found at metaphase I of meiosis (30–80%) compared with that observed for normal males (0–10%).

The results of assays using skin and heart fibroblasts (see Table 1) indicate that STS activity does not reflect the different X chromosome dosage in males and females. A similar conclusion is suggested by observations of liver extracts from mice obtained from crosses involving *Sxr*, XX and XY individuals having similar STS levels irrespective of their phenotypic sex. Estimations of glucose 6-phosphate dehydrogenase (G6PD) of fibroblast preparations, although more variable than those for STS, showed no significant differences in the levels of this enzyme for the male and female preparations. The lack of detectable differences in the male and female levels of STS makes it unlikely that the gene for steroid sulphatase lies on a non-inactivated region of the X chromosome in mice. This conclusion is of particular interest because it has been suggested, on the basis of an observed proportionality of STS levels to the number of X chromosomes present in cultured fibroblasts, that the STS locus in the wood lemming is part of a region that escapes inactivation⁵, similar to that found in humans. Our observations suggest that the genetic status of STS may have changed during mammalian evolution. It would not have been remarkable to have observed such a change between rodents and man, but it is surprising that such a change should have occurred between the relatively closely related species of mouse

Table 1 STS activity in mice of different sex chromosome constitution

Mouse type	No. of mice	STS activity	Total no. of observations
Embryo skin fibroblasts:			
XX, female	12	80 (5)	48
XY, male	11	91 (7)	44
Adult heart fibroblasts:			
XX, female	6	117 (4)	24
XY, male	6	124 (7)	24
Adult liver extracts:			
XX, female	6	420 (42)	12
XX <i>Sxr</i> , male	6	410 (58)	12
XY, male	6	437 (39)	12
XY <i>Sxr</i> , male	7	378 (38)	14
G6PD activity			
Embryo skin fibroblasts:			
XX, female	5	0.243 (0.07)	10
XY, male	5	0.213 (0.06)	10

Fibroblast cultures were established from minced, eviscerated embryos. The trypsinized tissues were plated in medium containing 15% bovine fetal serum and the attached cells passaged at least once before collection. Cultures from adult hearts were established in a similar way from minced and trypsinized material. Mean STS activities (with standard errors shown in parentheses), are expressed as pmol dehydroepiandrosterone sulphate (DHEAS) cleaved per mg protein per h. To obtain extracts for determination of STS activity from cultured fibroblasts, semi-confluent cells were washed twice in phosphate-buffered saline, resuspended in 50 mM HEPES (buffered to pH 8.0 with KOH) and sonicated (40 s). Liver extracts were obtained by homogenization in 5 ml of 100 mM HEPES, pH 8.0, per g of liver. The homogenate was centrifuged at 3,000g for 10 min and the supernatant sonicated for 60 s. For the STS assays tritiated DHEAS (specific activity 24 mCi μmol^{-1} ; NEN) was diluted with cold DHEAS to a specific activity of 50 $\mu\text{Ci} \mu\text{mol}^{-1}$, then extracted twice with diethyl ether to remove free dehydroepiandrosterone; 20 nmol of the diluted DHEAS were incubated at 37 °C for 2 h with 100 μl of sonicated solution and 76 μl of 50 mM HEPES, pH 8.0. Fifty microlitres of 1 M NaOH were added at the end of the incubation, followed by extraction twice with 3 vol. of ether. The ether phase was evaporated to dryness, and radioactivity determined by scintillation counting. Blank controls contained heat-inactivated cell and liver extracts. STS activity was determined for each mouse by taking the mean of either two independent fibroblast cultures or of two independent assays on liver extracts. Mean protein concentration in the fibroblast homogenates was 10.6 mg ml^{-1} and in the liver extracts 26.0 mg ml^{-1} . Commercial samples of G6PD (Boehringer Mannheim) were used to check the enzyme assay system, which was based on that recommended by the manufacturers. Results expressed in $\mu\text{mol per min per mg protein} (\pm \text{s.e.})$.

and wood lemming. Based on the evidence presented to date, however, this conclusion seems warranted. Data must be obtained on the genetic status of STS in other mammals before definite conclusions can be drawn about the evolutionary stability of non-inactivated genes on the X chromosome.

Although the results clearly indicate a different pattern of expression of steroid sulphatase in the mouse compared with that reported for human and wood lemmings, the molecular mechanism(s) underlying the observation remains to be elucidated. If one assumes that the enzyme activity assayed in mouse extracts is homologous to STS in man, there are two possible explanations: either the STS gene is X-linked in the mouse, but is subject to the normal pattern of X-inactivation, or the locus is autosomal. One further possibility that cannot be excluded formally is that STS loci exist on both the X and Y chromosomes in mice; however, Gartler and Rivest³ recently presented evidence supporting X-linkage based on studies of STS levels in oocytes of XX and XO mice.

There have been reports of differences between inbred mouse strains in STS levels which are determined autosomally⁶ and our own unpublished observations have also identified a putative, autosomal locus involved in the regulation of STS

expression. Nevertheless, the simplest explanation consistent with our observations is that the spread of inactivation along the short arm of the human X chromosome does not extend far enough to embrace the STS locus, but that in the mouse inactivation is more extensive. Recently, Migeon *et al.*⁷ have shown that although the STS locus is expressed when present on an inactive human X chromosome, the escape from inactivation may be incomplete. It remains to be demonstrated whether such partial inactivation extends to the remainder of the Xp terminal region which is thought to carry also a gene for the Xg blood group and a locus necessary for the expression of a serologically defined male-specific antigen². Genetic studies on mice have not revealed any regions of the X chromosome which escape inactivation. The most distal loci (hypophosphataemia and cream) and the most proximal (sparse fur) on the mouse chromosome are X-inactivated and, therefore, if there is a terminal non-inactivated region, it must lie beyond the boundaries of the presently established genetic map⁸. Furthermore, recent observations on XX *Sxr* mice which carry *Sxr* on the distal end of one X chromosome indicate that inactivation can spread to encompass the *Sxr* locus^{9,10}. Consideration of XO individuals also suggests that there may be differences in the

organization and activation of X-linked genes of mice and man. Although XO mice are normal in appearance and are fertile, human XO individuals are abnormal and infertile. The abnormalities observed in humans could be explained in part if normal development is dependent on the presence of two copies of X-linked genes which escape inactivation, in which case the apparent normality of the mouse XO would imply that such genes are not on the mouse X chromosome, or are effective at a single dosage.

Whereas the wood lemming and human X chromosomes are metacentric, that of the mouse is acrocentric. It may be significant that in humans all of the genes which are believed to escape inactivation are at the tip of the short arm of the chromosome, a position which has no equivalent in the mouse. Detailed comparative studies on the organization and expression of mammalian X-linked genes may therefore be valuable in elucidating the X-inactivation mechanism.

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Nucleotide sequence of epidermal growth factor cDNA predicts a 128,000-molecular weight protein precursor

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Epidermal growth factor (EGF) has a profound effect on the differentiation of specific cells *in vivo*, and has been shown to be a potent mitogenic factor for a variety of cultured cells, of both ectodermal and mesodermal origin (see ref. 1 for review). This 53-amino acid polypeptide of known sequence² contains six cysteine residues³, which are thought to form three intrachain disulphide bonds⁴. Urogastrone, a polypeptide bearing anti-gastric secretory activity isolated from human urine⁵, which is presumably synthesized in submandibular and Brunner's glands^{6,7}, shares extensive sequence homology (70%) with EGF and may represent the human EGF equivalent. Here we present the sequence of a mouse EGF cDNA clone, which suggests that EGF is synthesized as a large protein precursor of 1,168 amino acids. Our data indicate that the discrepancy between EGF levels in male and female mouse submaxillary glands (MSGs) is due to different EGF mRNA levels in these tissues, and suggest that precursor EGF processing may differ from that described previously for other polypeptide hormones.

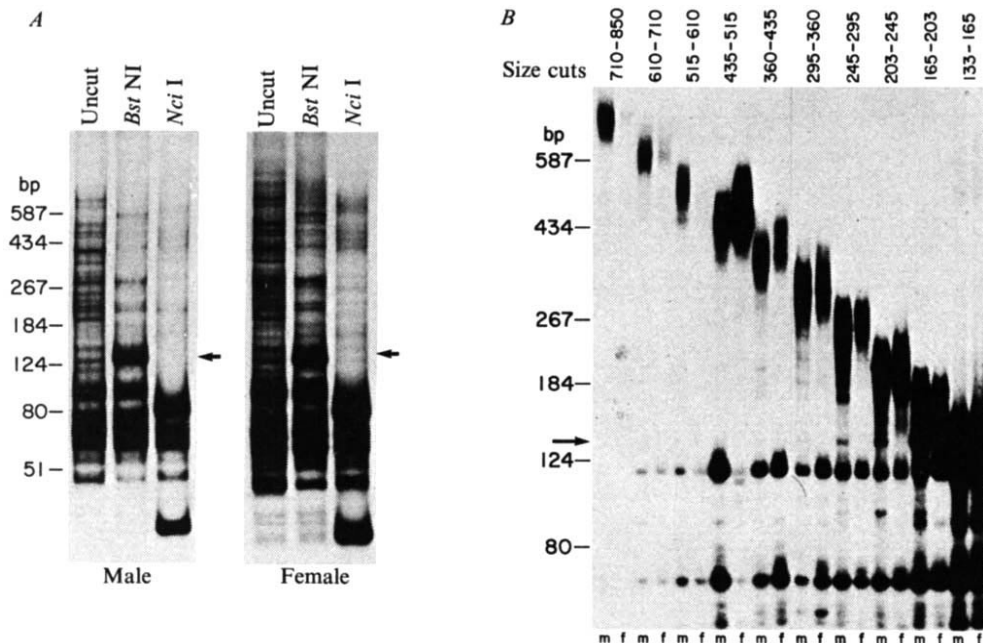
The carboxy-terminal region of EGF contains an amino acid sequence (Trp-Trp-Glu-Leu) which can only be coded for by four possible nucleotide sequences. Thus, four specific undecamers were synthesized to complement these coding sequences (see Fig. 1 legend). The synthetic oligonucleotides were pooled, 5'-end labelled, and used to prime cDNA synthesis on mRNA preparations from male and female MSGs. The male MSG contains significantly more EGF protein than the female MSG¹; assuming that this was due to differences in mRNA levels, we used comparison of male and female MSG cDNA

in an attempt to facilitate the identification of EGF cDNA. Acrylamide gel analysis showed that our oligonucleotides unexpectedly primed cDNA synthesis from a large variety of templates present in male and female MSG mRNA, and no obvious pattern difference between male and female double-stranded (ds) cDNA could be detected (Fig. 1A). It was possible that either the EGF mRNA was a minor component of the male mRNA, or that the difference in protein levels between the male and female glands was not due to differences in mRNA levels, as we had assumed. Therefore, restriction enzyme cleavage analysis was done to resolve this point. On the basis of the EGF amino acid sequence, we determined all possible restriction fragment lengths which could be generated by priming cDNA synthesis with our specific oligonucleotides and digestion of the resulting ds cDNA with the restriction endonucleases *Bst*NI and *Nci*I. These enzymes were chosen because the amino-terminal region of EGF contains a Pro-Gly dipeptide which is coded for by CCNGGN (N = A, T, G or C) and represents a restriction site for either *Bst*NI (CC^T_AGG) or *Nci*I (CC^C_GGG). Given this known cleavage site and the distance between the cleavage site and the primer, it could be calculated that the maximum size of an EGF cDNA fragment generated by *Bst*NI or *Nci*I cleavage would be 144 bp. The generation of shorter fragments (*Bst*NI: 23 and 79 bp; *Nci*I: 23 and 43 bp) was predicted to be of a lower but finite probability. Figure 1A shows that fragments of the predicted possible size were generated but were not unique to the male MSG cDNA. To test for the presence of EGF mRNA in the female gland, we determined the nucleotide sequence of the common ~145-bp *Bst*NI fragment. The sequence did not contain any EGF coding information (data not shown), but contained a *Hae*III cleavage site, which could not be present in mature EGF coding sequences. Therefore, *Hae*III digestion was used to remove the common ~145-bp fragment. To improve resolution we electrophoresed primed, *Hae*III-digested ds cDNA first on an acrylamide gel, cut the gel into slices, and eluted each slice individually. Each cDNA fraction was then digested with *Bst*NI or *Nci*I and analysed on another acrylamide gel. Autoradiography of the dried gel showed that a ~145-bp *Hae*III/*Bst*NI fragment was indeed present in the cleaved male cDNA and not detectable (<10%) in the female (Fig. 1B). The fractions that yielded the

Fig. 1 *A*, Comparison of ds cDNA synthesized by priming male or female MSG polyadenylated RNA with a pool of four synthetic undecamers. *B*, Comparison of restriction endonuclease digestion products of various size fractions of *Hae*III-digested male and female MSG ds cDNA.

Methods: *A*, The synthetic oligonucleotide pool (3'-ACCACCTCGA-5') (140 pmol) was phosphorylated to a specific activity of $\sim 10^6$ c.p.m. pmol⁻¹. After phenol-chloroform extraction, the ³²P-labelled primers were ethanol-precipitated with 6 μ g poly(A)⁺ RNA, which had been isolated from fresh mouse (Swiss-Webster) submaxillary glands by the guanidine thiocyanate-CsCl procedure¹⁹ followed by oligo(dT)-cellulose chromatography. The pellet was dissolved in 20 μ l of 100 mM KCl and the mixture annealed by incubating for 3 min at 90, 68, 42, 37 and 22 °C. cDNA was synthesized

by diluting the RNA-primer mixture into a 40- μ l reaction mixture containing 50 mM Tris pH 8.3, 50 mM KCl, 10 mM MgCl₂, 10 mM dithiothreitol, 0.5 units per μ l RNase inhibitor (BRL), 500 μ M each of dATP, dGTP, TTP and dCTP, and 25 units of reverse transcriptase (Beard). Incubation was at 37 °C for 1 h. The reaction mixture was boiled (3 min), quenched (1 min) at 0 °C, centrifuged (2 min) at 12,000g, and the supernatant was then diluted to 40 μ l with water. ds cDNA was synthesized overnight at 12 °C with 10 units of *Escherichia coli* DNA polymerase I (Klenow fragment). After treatment with pancreatic ribonuclease A (100 μ g ml⁻¹ at 37 °C for 60 min), phenol-chloroform extraction, and ethanol-precipitation, one-third of the resulting ds cDNA was digested separately with *Bst*NI or *Nci*I. These digests, as well as uncut cDNA, were analysed by electrophoresis on an 8% polyacrylamide gel followed by autoradiography of the dried gel. Only cDNA species which incorporated the radiolabelled primer were detected on the gel. The arrow indicates the position of the predominant ~ 145 -bp restriction digestion product which was eluted from the dried gel for Maxam-Gilbert sequencing⁷. Equivalent slices from lanes containing male and female samples were soaked in 500 mM ammonium acetate, 10 mM Mg-acetate, 1 mM EDTA, 0.1% SDS at 68 °C for 1 h to rehydrate the gel, then at 37 °C overnight after crushing the gel. The eluate was recovered by centrifuging the gel mixture through the Quik-Sep column (Isolab) with a scintered glass filter. The eluate was phenol-chloroform-extracted and ethanol-precipitated. Approximately 50% of the counts were recovered. *B*, ³²P-labelled oligonucleotide primers (140 pmol) were annealed to 12 μ g of male or female poly(A)⁺ RNA and ds cDNA synthesized as described for *A*. After ribonuclease A treatment, phenol-chloroform extraction and ethanol precipitation, the ds cDNA was digested with 10 units of *Hae*III for 1 h at 37 °C. The extraction, precipitation and digestion steps were repeated. The digests were fractionated on a 2.4-mm thick 6% polyacrylamide gel; each lane was cut into 10 0.5-cm slices in the size range 133–850 bp. The slices were crushed and eluted as described for *A*. The ethanol-precipitated cDNA was dissolved in 60 μ l H₂O; 10- μ l aliquots (5,000–20,000 c.p.m.) from each fraction were digested in 20- μ l reaction volumes with 5 units each of *Bst*NI or *Nci*I. Digestion products were electrophoresed on a 40-cm long, 1.2-mm thick, 6% polyacrylamide gel overnight at 150 V. The gels were dried and exposed to X-ray film overnight with an intensifying screen (Cronex Lightning Plus). ³²P-labelled pBR322 *Hae*III fragments were used as size markers. Only the *Bst*NI digestion is shown. The size cut range (bp) for each pair of lanes is indicated at the top. m, Male MSG poly(A)⁺ RNA used as template; f, female. The arrow indicates the ~ 145 -bp male-specific *Bst*NI fragment. Only those fragments which contain the radiolabelled primer can be detected on this autoradiograph.



~ 145 -bp male-specific fragment were in the size range 150–200 bp. cDNA molecules present in these fractions were cloned as described in Fig. 2 legend; 2,400 clones were screened with the oligonucleotides originally used for cDNA priming⁸ in addition to an oligonucleotide pool (eight tetradecamers) derived from the EGF sequence between amino acid 20 and 24

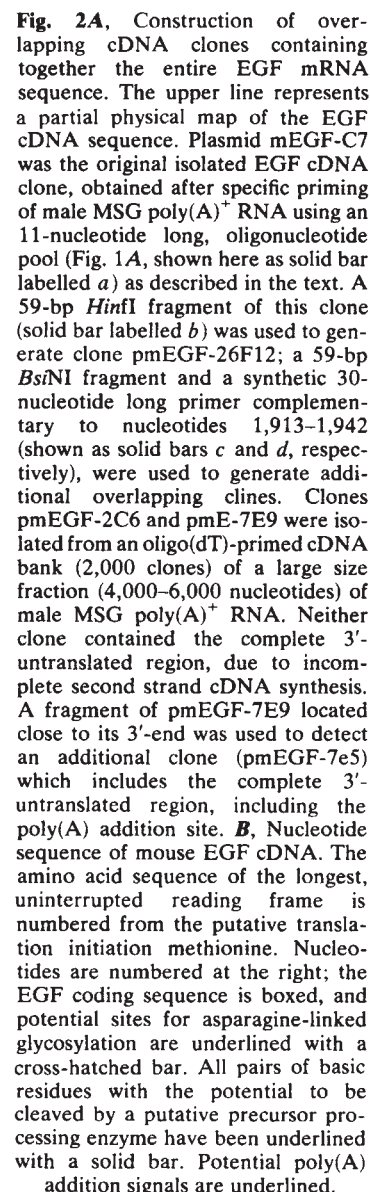
(3'-ACG^ATACGT^AGTA^AGCT-5'). Four clones hybridized to both primer pools and were later found to be identical; they each contained EGF coding sequences. Clone pmEGF-C7 was sequenced in its entirety by the Maxam-Gilbert method⁹. Its 171-bp DNA insert begins at the 3' end with the complementary primer sequence TGGTGGGAGCT, and ends at the 5' end with a *Hae*III site as planned.

Northern blot analysis showed, to our surprise, that the EGF probe hybridized to a male MSG RNA species $\sim 4,700$ nucleotides long (data not shown). Considering that the EGF protein requires only 159 nucleotides to encode it, the existence of a mRNA 30 times this size was a remarkable finding.

DNA restriction fragments, synthetic DNA and oligo(dT) were each used as specific cDNA synthesis primers to obtain cDNA molecules containing the entire EGF mRNA sequence (Fig. 2). The nucleotide sequences of seven overlapping clones (Fig. 2A) were completely determined⁹ and are shown in Fig. 2B.

Examination of the 4,706-bp nucleotide sequence (Fig. 2B) revealed that one translational reading frame could code for an uninterrupted sequence of 1,168 amino acid residues. The 53 amino acids of mouse EGF were found to be embedded within this sequence; the 3,504 nucleotides encoding the presumptive EGF precursor protein are flanked by a 892-nucleotide long 3'-untranslated region and by an unusually high number (309) of untranslated nucleotides at the 5'-end. The 3'-untranslated region contains two AATAAA sequences, thought to be important for polyadenylation of mRNAs¹⁰; either of these sites may be used for polyadenylation¹¹. The number of nucleotides absent from our 5' sequence (~ 30 –40) was determined in a primer extension experiment (data not shown).

The open reading frame that includes the EGF amino acid sequence starts with an initiation codon ATG, three nucleotides downstream from an in-frame termination codon (TAA). The initiator methionine is followed by a stretch of 29 amino acids which are rather hydrophobic and closely resemble a signal peptide. The potential signal peptidase cleavage site is unknown; however, as cleavage usually occurs subsequent to a small amino acid¹², and given the average length of signal peptides (~ 15 –30 residues), amino acids Ile 23, Val 26 and Trp 29 represent possible candidates for the amino-terminal residue of pro-EGF. The sequence of the mature EGF protein



The EGF precursor protein contains five potential sites for asparagine-linked glycosylation at residues 111, 410, 810, 944 and 1,116, which are evenly distributed throughout the protein. Two regions of the precursor are particularly cysteine-rich: 330–470 and 750–1,020, the latter region containing mature

320 DPELLKQRGR--PQ--RFGLC--ERDPKSHSSAEGYTLSDRKVC
 EDVNEC-ATONHGC--TLGC--ENTPGSYHETPTGFVLDPGKQC
 HELVSC-PGNVSKC--SHGC--VLTSDGPRKICPAGSVLGRDGRK
 TGCSPDNGGC--SGLC-LPLRPGSWEKDFPGLQSDRKSC 482
 745 KPGADPC-LYRNGGC--EHTQEQLSG-TARELEREGFVKAMDGMK 786
 835 GMNVEDDC--EPGGGSH-ARCV-SDGE-TAEQQLKGF-ARDGNLC
 SDIDEK-VLARSDPSTSSKINTEGGYV--ERCSEGY-EGDGTSC
 FDIDEK-ORGAHNC-AENACTINTEGGYN--ETKAGRP--SSPGRSC 958
 975 DRNSY-PGEPSPSYDGYE-LNGGVEMHIESLDSYTENVIGY-SGD-R- 1,018

Fig. 3 Repeated sequence homologies within the EGF precursor and their relationship to sequences of other proteins. Amino acid positions are shown at the beginning and end of the partial sequences. Dashes indicate gaps introduced to achieve optimal alignment of cysteine residues. Shading indicates an amino acid which occurs at the same position in at least one other repeat unit.

EGF sequences. Comparison of the EGF precursor sequence with all proteins listed in the Dayhoff *Atlas of Protein Sequence and Structure*²⁰ did not reveal any extensive homology with any other known protein sequence, including the EGF binding protein and nerve growth factor.

Several other polypeptide hormones are synthesized as large precursor proteins, including proenkephalin A¹⁴ and B¹⁵, proopiomelanocortin¹⁶ and procalcitonin¹⁷. In each of these cases, active polypeptide sequences are usually bounded by the pairs of basic amino acid residues Lys-Arg, Lys-Lys or Arg-Arg; it is at these sites that proteolytic processing occurs. While pairs of these basic residues are found in the putative EGF precursor sequence, they do not flank the mature EGF protein. Processing of EGF appears to occur between Arg-Asn at the amino-terminus and Arg-His at the carboxy-terminus. In each case one basic amino acid remains part of the EGF polypeptide.

Frey and co-workers¹⁸ reported the existence of a 9,000-M_r biosynthetic precursor of EGF; these workers provided evidence which suggested that the precursor contained a carboxy-terminal polypeptide extension. The 9,000-M_r polypeptide could be an intermediate cleavage product, terminating perhaps at one of several basic amino acid residues located between amino acids 1,060 and 1,080 of the EGF precursor (Fig. 2B).

It is unlikely that such a large precursor polypeptide codes for just a small polypeptide hormone, and the presence of multiple basic dipeptides in the sequence suggests that additional active polypeptides may also be encoded by it. Analysis of the cysteine-rich areas described above revealed an interesting regular spacing pattern of the cysteine residues common to both regions. The boundaries of the sequence repeat units are not readily apparent, but the sequence could be aligned to reveal nine groups, eight of which displayed homologous cysteine residue positions (Fig. 3). Some sequence gaps had to be introduced to achieve optimal alignment, but the high degree of similarity between the nine sequence units which include part of the mature EGF sequence is evident. We do not know whether these regular sequential patterns are structurally necessary to maintain a conformation essential for the correct processing or intracellular traffic of the EGF precursor. Alternatively, these regular cysteine arrangements (or portions thereof), with variable but related spacer sequences, could reflect independent polypeptides having different, and thus far unknown, functions within the EGF precursor.

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Nucleotide sequence of cloned cDNA of human c-myc oncogene

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Like other transforming genes of retroviruses, the v-myc gene of the avian virus, MC29, has a homologue in the genome of normal eukaryotic cells. The human cellular homologue, c-myc, located on human chromosome 8, region q24-qter (refs 1, 2), is translocated into the immunoglobulin heavy-chain locus on human chromosome 14 (ref. 3) in Burkitt's lymphoma^{1,4,5}, suggesting that c-myc has a primary role in transformation of some human haematopoietic cells. In addition, c-myc is amplified in the human promyelocytic leukaemia cell line, HL60 (refs 6, 7) which also contains high levels of c-myc mRNA⁸. Recently, Colby *et al.*⁹ reported the nucleotide sequence of the human c-myc DNA isolated from a genomic recombinant DNA library derived from human fetal liver¹⁰. This 4,053-base pair (bp) sequence includes two exons and one intron of the myc gene, and the authors have suggested the existence of a human c-myc mRNA of 2,291 nucleotides that has a coding capacity for a protein of molecular weight (M_r) 48,812. We have approached the problem of accurately defining the characteristics of the human c-myc mRNA and c-myc protein by determining the sequence of the c-myc cDNA isolated from a cDNA library prepared from mRNA of a clone of the K562 human leukaemic cell line¹¹. K562 cells are known to contain c-myc mRNA which is similar in size to the c-myc mRNA of other human cell types⁸. We report here the sequence of 2,121 nucleotides of a human c-myc mRNA and demonstrate that its 5' noncoding sequence does not correspond to the sequence of the reported genomic human sequence. However, our data confirm that the intact human c-myc mRNA can encode a 48,812-M_r protein with a sequence identical to that reported by Colby *et al.*⁹.

The restriction map of the human c-myc cDNA and strategy of sequencing are shown in Fig. 1A. Figure 2 shows the 2,121-bp nucleotide sequence of the human c-myc cDNA as determined

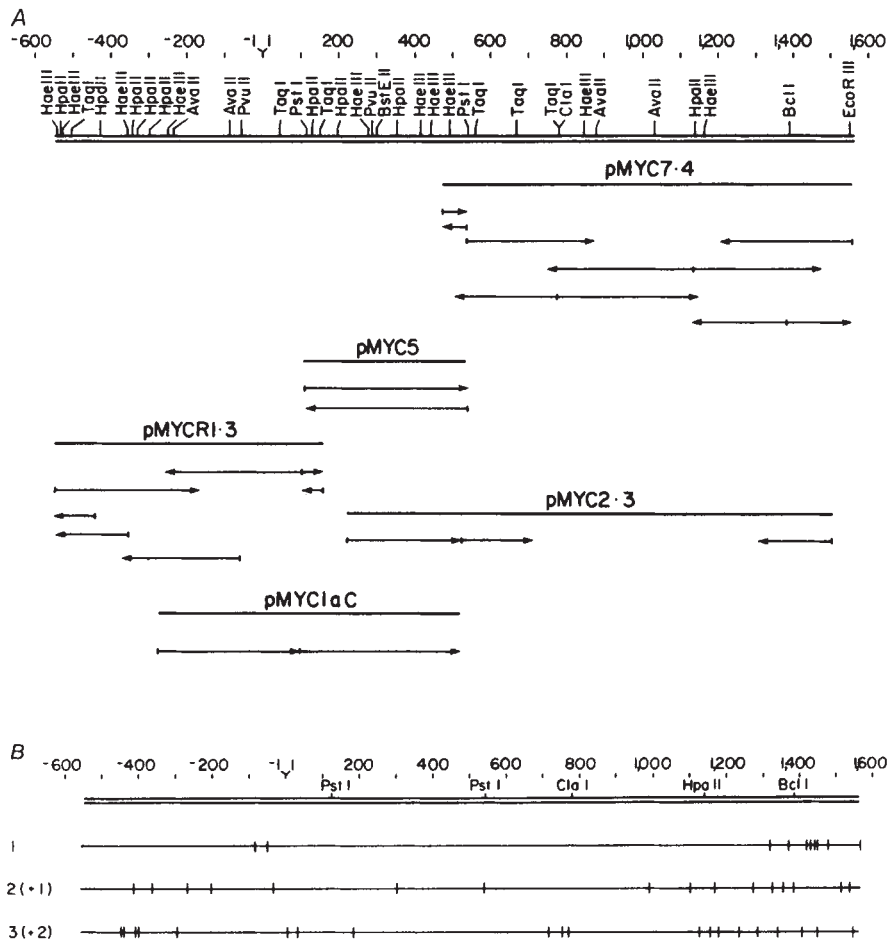
Fig. 1 A, Restriction endonuclease map of human *c-myc* cDNA clones and strategy of sequencing. The solid bars represent the *c-myc* cDNA inserts. Horizontal arrows indicate the direction and length of each fragment sequenced. Nucleotide residues are numbered in the 5'→3' direction in the message strand, beginning with the first residue in the coding region for *c-myc*. The nucleotides on the 5' side of residue 1 are indicated by negative numbers. **B**, Translational reading frames of human *c-myc* cDNA. Vertical bars denote stop codons. The open reading frame for *c-myc* (frame 1) is the frame containing the first translation initiation signal.

Methods: A, K562(S) (ref. 11) total cytoplasmic RNA was isolated¹⁷ and further purified by chromatography on oligo(dT)-cellulose¹⁸. The yield was 0.12–0.14 g poly(A)-containing RNA per 10⁶ cells. This mRNA served as a template for AMV reverse transcriptase to prepare cDNA¹⁹ primed by oligo(dT), which was then converted to double-stranded (ds) DNA using the same enzyme²⁰. After treatment of the ds cDNA with *Aspergillus oryzae* S₁ nuclease²¹, homopolymeric poly(dC) tails were added to the 3'-ends by using calf thymus terminal deoxynucleotidyl-transferase²². The poly(dC)-tailed ds cDNA was fractionated by agarose gel electrophoresis²³ and sequences with an average length >1,000 bp were annealed to poly(dG)-tailed, *Pst*I-cleaved pBR322 plasmid

DNA. *Escherichia coli* HB101 was transformed by the recombinant plasmids²⁴ and screening for tetracycline-resistant cells yielded >2 × 10³ colonies per ng of ds cDNA inserted. The tetracycline-resistant clones were screened by the Grunstein-Hogness colony hybridization procedure²⁵, using as a probe a 32P-labelled 1.4-kilobase (kb) *Cla*I-*Eco*RI fragment of λLMC41, a recombinant Charon 4A phage, containing the human *c-myc* gene²⁶. ³²P labelling was performed by nick-translation²⁷. Hybridization and washing of blots were done essentially as described elsewhere²⁸. Ninety-one *c-myc*-containing clones were identified after screening of 5.7 × 10⁵ recombinants. One of these positives clones (pMYC7.4, which contains a 1.2-kb insert) was cloned into the M13 phage vectors mp8 and mp9 (ref. 29) and then sequenced using the chain termination technique³⁰. Restriction mapping indicated that this insert is a copy of *c-myc* mRNA that is incomplete with respect to both the 3'- and 5'-ends. Clones containing additional 5'-end sequences were selected using a full-length mouse cDNA clone¹⁴ as probe. Five cDNA clones (pMYC7.4, pMYC2.3, pMYC5, pMYC1.3 and pMYC1aC) containing extensive overlapping sequences were used to determine the sequence of 2,121 nucleotides of *c-myc* mRNA. In the figure only those segments that were fully sequenced are shown. Several other clones were used to confirm the findings in specific areas of the DNA sequence.

from the five recombinant clones analysed. The reading frame of *c-myc* has been deduced from the position of the termination codons in the three reading frames (Fig. 1B). The nucleotide sequence from position -19 to the 3'-end of the cDNA clones is identical to that reported by Colby *et al.*⁹ when allowance is made for the removed stretch of sequence consisting of the single intron reported for the genomic sequence. Our findings confirm the positioning of the splice sites of the intron described⁹. However, comparison of the cDNA and genomic DNA sequences upstream to the identically positioned Met initiation signal reveals a lack of homology with any stretch of the reported sequence⁹, beginning 20 bases upstream (Fig. 3). The transition between the homologous sequence and the different sequence was marked by the CAGCAG sequence in one of three clones examined (clone pMYC 3.27). In the other two cDNA clones we noted the presence of a single CAG triplet (nucleotides -13 to -15 in Fig. 3) exactly at the point of divergence between the genomic DNA sequence and cDNA sequence.

The amino acid sequence predicted from the open reading frame of K562 mRNA (shown in Fig. 2) is identical in every detail to that previously reported for the human fetal liver⁹. This sequence could encode a protein with a molecular weight of 48,812. As reported by Colby *et al.*⁹, the amino sequence



differs from that of the *v-myc* of avian retrovirus MC29 (ref. 12) by the insertion of 18 and 22 amino acids and one deletion of 11 amino acids relative to the viral gene. A termination codon has been found at position -49 to -51 upstream from the proposed translation initiation signal. Moreover, no other ATG codon as an alternative translation initiation signal was found in any of the three reading frames in the first 600 bases of the sequence.

Our results demonstrate the complete absence of homology between the 5'-end sequence of the human *c-myc* mRNA obtained from a cDNA library of human leukaemia cells, and the 5'-end sequence derived from a normal human fetal liver library⁹. This difference can be explained by a misidentification of a splicing acceptor site located at position 986–1,002 of the genomic sequence. The sequence in that position (TGCCCCGCTCCAGCAGC) contains a polypyrimidine-rich stretch followed by CAGCAGC, and it therefore has the characteristics of an acceptor consensus sequence for a splicing site¹³. Moreover, the repetition of the nucleotides CAG could result in ambiguity in the position of the splicing site¹³. This ambiguity would explain our finding of two CAG triplets in one of the cDNA clones (pMYC 3.27). If ambiguity exists at the acceptor site, and the donor sequence is spliced after a CAG triplet¹³, either one or two CAG triplets could result in

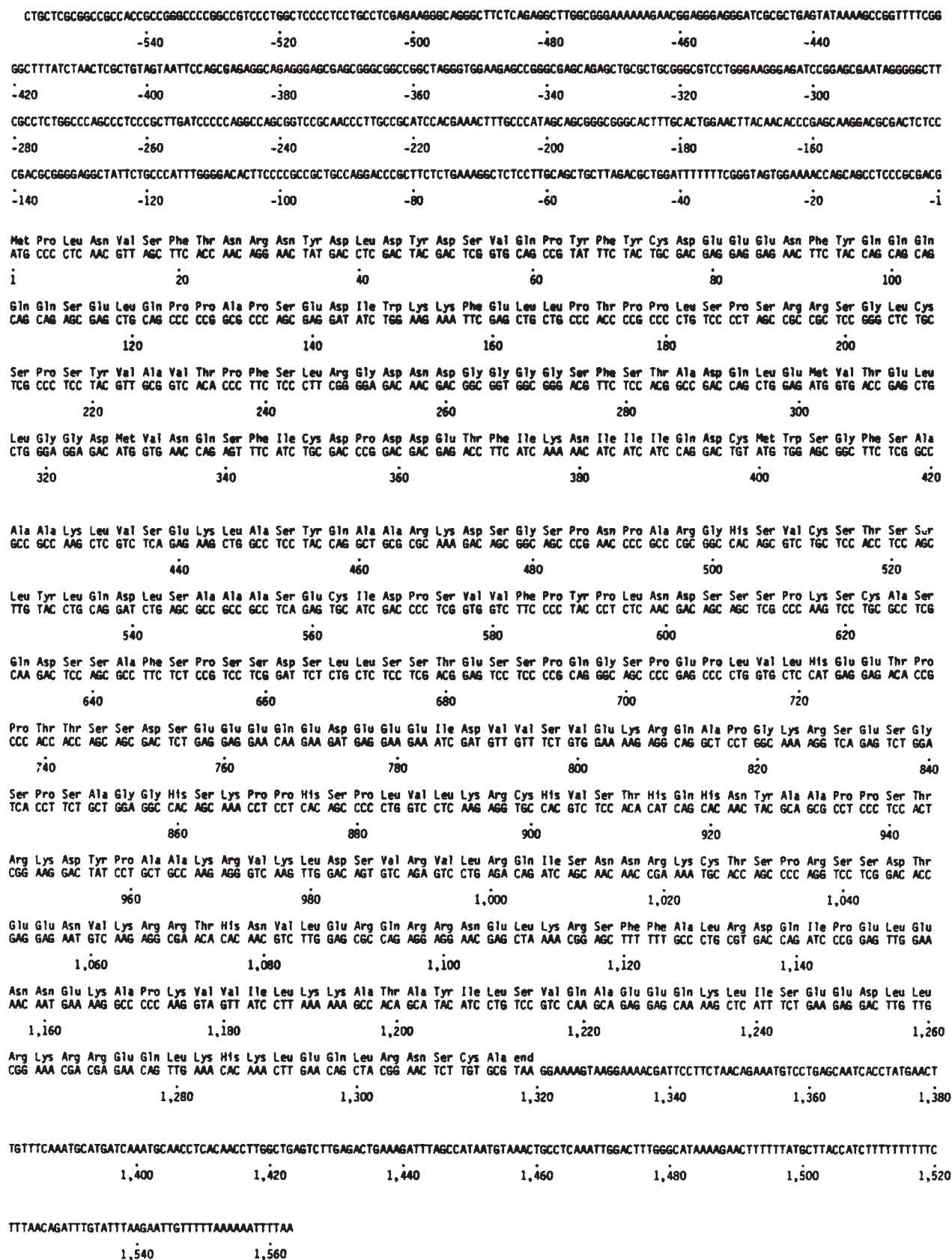


Fig. 2 Nucleotide sequence of the *c-myc* cDNA. The Sanger 'dideoxy' method³¹ was used for sequence determination. After restriction endonuclease treatment, fragments of the plasmid inserts were subcloned into M13 mp8 or M13 mp9 (ref. 2). Clone pMYC7.4, pMYC2.3, pMYC5, pMYIaC and pMYCR1.3 sequences that represent portions of the same *c-myc* gene product, were joined into a continuous 2,121-nucleotide sequence. The nucleotide numbers are indicated below the sequence (see Fig. 1 legend). The amino acid sequence of the putative *c-myc* protein deduced from the nucleotide sequence is indicated from nucleotides 1 to 1,320.

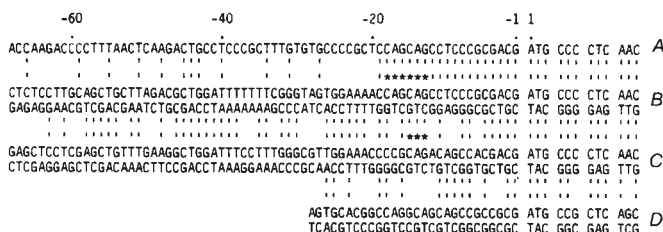


Fig. 3 Comparison of *myc* DNA sequences at the transition between coding and 5'-noncoding region. ***, Splicing site; *****, proposed alternative splicing sites. Vertical bars indicate areas of homology. A, Human genomic sequence¹⁷; B, human cDNA sequence; C, mouse cDNA sequence¹⁴; D, MC29 DNA sequence¹².

the processed mRNA, as we in fact observed. We have recently compared a mouse cDNA *c-myc* clone¹⁴ with a mouse genomic *c-myc* clone and have reported the presence of a second intron in the *c-myc* mouse genomic sequence. A comparison of these sequence data with the data on the human *c-myc* sequence indicates that the acceptor splicing site resides exactly at the site where the human cDNA sequence diverges from the genomic DNA sequence.

The presence of a putative promoter signal, a TATA box and a cap signal in the genomic clone suggests the possibility that in some cases a *c-myc* mRNA with the characteristics described by Colby *et al.*⁹ is synthesized. This would predict that multiple initiation sites and splicing sites coexist in the *c-myc* gene. A similar situation has already been reported for the α -amylase gene of the mouse¹⁵, where different transcription initiation sites and splicing sites generate mRNAs having different 5' noncoding regions but identical protein-coding regions in salivary gland and liver. Analysis of the mRNAs of different cell lineages, and of Burkitt's lymphoma cells, with *c-myc* rearrangements, using as probes both the sequence present in the 5'-end of the cDNA *c-myc* clones and the protein coding sequence, will allow us to test this possibility.

A second possible explanation of the difference in the 5' noncoding region is that a different rearrangement of the 5'-end of the *c-myc* genome occurs during development or differentiation in normal cells. A third possibility is that the 5'-end sequence of human leukaemic cells might be abnormal and unrelated to the normal *myc* gene but caused by genomic rearrangement during malignancy. This last possibility should be considered in view of the findings in human Burkitt's lymphoma and mouse plasmocytoma¹⁴.

Despite the differences in more than 600 nucleotides of the 5'-end sequences of the *c-myc* cDNA reported by us and by Colby *et al.*⁹, the size and sequence of *myc* protein are identical in erythroleukaemic cells and fetal liver. Both sequence analyses indicate that the human *myc* mRNA is capable of encoding a protein with an apparent molecular weight of 48,000. This protein, therefore, has a molecular weight similar to that encoded by the *v-myc* portion of the MC29 fused *gag-myc* protein. In the case of K562 human leukaemia cells, the protein-coding sequence is preceded by a long 5' noncoding sequence of 600 nucleotides. Accurate determination of the length of the 5' noncoding sequence, however, awaits studies using the primer extension technique¹⁶. The evidence regarding a relatively long noncoding sequence at the 5'-end of the *c-myc* mRNA is based on the following observations: (1) the first identifiable translation initiation signal in any frame is 600 nucleotides from the beginning of the sequence and is used as the translation initiation signal of the putative *myc* protein; (2) there are at least two termination signals in every reading frame upstream from the protein-coding region, relatively close to the Met initiation signal. Termination signals in the reading frame of the 5'-untranslated region have also been reported for the α -amylase gene¹⁵; (3) the initiation signal, the termination signal 49 bp 5' of the translation initiation signal, and the lack of other ATG codons in the upstream sequence occur in

independently isolated cDNA clones, ruling out the possibility of incorrect positioning of such signals.

Our results emphasize the need for more information about the sequence of the 5'-noncoding region in *c-myc* mRNAs of different cell lineages. This should help to elucidate the mechanism controlling the expression of the *c-myc* gene in differentiation and oncogenesis.

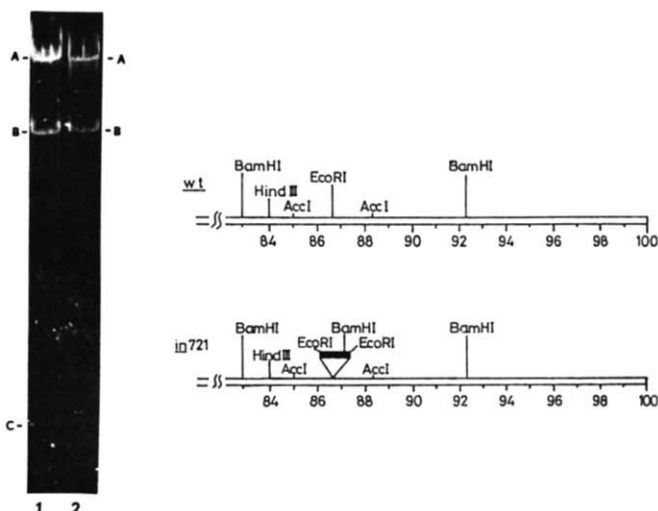
We thank Dr P. Curtis for helpful suggestions during the preparation of the K562(S) library and for critical reading of the manuscript; D. Donaldson, J. Ming and J. Sorrentino for technical help; and M. Hoffman for editorial assistance. K.B.M. is a NIH research career development awardee. This work was supported by NCI grants CA 10815, CA 16685, CA 24273, CA 25875, AI 00416 and GM 26939.

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Erratum

In the article 'Hinf family: a novel repeated DNA family of the human genome' by Y. Shimizu *et al.*, *Nature* **302**, 587-590, an incomplete version of Fig. 1 was used. The correct figure is shown below and will be available in reprints.

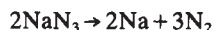


Thermal decomposition of solids

RAJESHWAR *et al.*¹ are probably the first to use differential scanning calorimetry (DSC) to study certain solid-state reactions in the presence of high electric fields within 100–400 kV m⁻¹. Shifts in temperature of the DSC peaks, changes of areas under individual peaks, or both were observed. The authors regarded these phenomena as kinetic effects of the field on thermal decomposition, and with this assumption discussed briefly the various mechanisms that may be responsible.

We would like to make two comments. First, we suggest that some of the reactions examined are not thermal decompositions enhanced by the field, but electrically-induced degradations accelerated by the rising temperature. Application of low fields such as 5–25 kV m⁻¹ is known to increase the rate of some thermal decomposition reactions (see, for example, refs 2–4). However, when high fields are involved charge carrier injection may occur and a novel type of 'electrochemical' mechanism may operate in ionic metallic compounds including sodium azide⁵. The electric fields applied in the experiments on NaN₃ have not been specified¹ but, if they are of the order of 10⁵ V m⁻¹ as reported for other substances, they would have been intense enough⁶ to cause partial chemical changes in a single crystal at room temperature by this mechanism. Rajeshwar *et al.*¹ have studied samples in powder form at elevated temperatures; the two differences in experimental conditions may be mutually compensating. In the case of AgN₃, the critical field for compacted powder is an order of magnitude greater than that for a single crystal, but the induction period for the onset of electron-hole injection decreases when the temperature is raised^{7,8}.

The second point concerns the 'pronounced' and 'drastic' increases of exothermicity observed during the decompositions of NaN₃ and KMnO₄. The former decomposes according to the equation



The extent of the above reaction, although dependent on the crystal size and temperature⁹, should reach completion in the experiments under discussion since the temperature was raised continuously to 623 K or higher. For metallic azides excepting the alkaline-earth compounds, the measured heats of reactions, Q , closely approach the corresponding heats of formation, ΔH_f . It would be interesting to compare the Q value obtained by the authors with the ΔH_f of NaN₃ which

is 322 kJ kg⁻¹. If Q (with electric field) exceeds ΔH_f , then we suspect that joule heating⁶ is responsible for their difference. The decomposition of KMnO₄ is complex in nature. From its DSC curves¹ we estimate Q at 0 and 200 kV m⁻¹ to be ~5 and 300 kJ kg⁻¹ respectively. In view of this drastic change in Q , we suggest that chemical characterization of the residues be carried out to clarify both the extent of decomposition and whether the reaction products are the same in both cases.

We also feel that experiments should be performed with the sample placed in

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an electric field applied through insulating electrodes, as has been done for silver azide⁵. This arrangement will prevent charge carrier injection and eliminate joule heating. Any kinetic or thermochemical effects found in such conditions can be truly attributed to the influence of electric field on thermal decomposition.

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Factors underlying falcon grating acuity

IN investigating falcon visual sensitivity to grating contrast¹, the contrast sensitivity functions of falcon and human observers were determined using stationary and phase changing gratings with a luminance of 40 cd m⁻². The study provides a qualitative comparison of falconiforme and human visual performance at that particular luminance, but the data do not warrant the quantitative analysis given for the possible factors underlying spatial acuity in the falconiforme deep fovea.

The extrapolated sensitivity functions show equal visual acuities (expressed in terms of cutoff frequency) for falcon and man. The author argues that the falcon shows an 'extraordinarily high' visual performance because the anatomical proportions of the subjects' eyes indicate a maximum falcon acuity three-quarters that of man. Since the observed human cutoff frequency was 40 cycles deg⁻¹, the expected proportional falcon cutoff frequency would be 30 cycles deg⁻¹ rather than the observed value of 40 cycles deg⁻¹. To account for this boost in acuity the theory that the spherical pit of the deep fovea acts as the divergent element in a telephoto lens system² is invoked. In this case, the spherical pit needs to provide an image magnification factor of 1.33. We feel that it is not necessary, nor in this case applicable, to suggest such a theory to explain falcon acuity when measured under the relatively low luminance of 40 cd m⁻².

To assess whether the falcon's acuity is 'extraordinarily high' or not, its behavioural acuity performance needs to be related to the potential anatomical performance of its own eye, rather than the behavioural performance of another species. The anatomical cutoff frequency of the falcon eye can be defined by the equation and data given in Hirsch's paper¹:

$$\nu = \frac{F}{d_{cc}\sqrt{3} \times 57.3} \times m$$

where ν is the cutoff frequency in cycles deg⁻¹, F is the falcon focal length of 9.1 mm, d_{cc} represents photoreceptor centre-to-centre spacing of 2 μ m, and m is the foveal magnification factor. Assuming no magnification, m is equal to 1. According to this equation the dimensions of the falcon eye allow for a cutoff frequency of 46 cycles deg⁻¹, which is above the behaviourally measured acuity of 40 cycles deg⁻¹. It appears that a measured acuity of 40 cycles deg⁻¹ is not 'extraordinarily high'. Note that the last measured response for stationary gratings was obtained at ~25 cycles deg⁻¹. The

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methods state that for each spatial frequency tested "a threshold was obtained only when the percentage of correct responses extended from 50% to ~90% or more" to allow a psychometric function to be drawn. This suggests the falcon did not respond to criteria for stationary frequency above ~25 cycles deg⁻¹, so perhaps 25 cycles deg⁻¹ is the appropriate cutoff frequency for the falcon, rather than the extrapolated value. In view of this ambiguity, it is unfortunate that the method of extrapolation was not stated. Irrespective of uncertainty regarding the actual cutoff frequency, it is unnecessary to invoke foveal image magnification to explain falcon acuity because even the higher extrapolated value is below the bird's anatomical cutoff frequency.

This particular falcon, the American kestrel, typically hunts in bright daylight conditions in luminances of $\geq 2,000$ cd m⁻². A simple explanation for the falcon acuity of 40 cycles deg⁻¹, rather than 46 cycles deg⁻¹, when tested at 40 cd m⁻², is that performance was luminance-limited³. The appropriate test of the magnification theory of the fovea is to measure spatial acuity under optimum luminance levels and to compare this behavioural cutoff frequency with that set by the anatomical constraints acting on the bird's eye.

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HIRSCH REPLIES—Dvorak *et al.* assert that since the observed cutoff frequency for the kestrel of 40 cycles deg⁻¹ is less than the 46 cycles deg⁻¹ expected from retinal anatomy, foveal magnification is not necessary. Their argument is flawed because they fail to consider accurately the extent to which low illumination limits kestrel vision.

In my study, human and kestrel cutoff frequencies were found to be nearly identical at moderate levels of photopic illumination, that is, an average of 40 cd m⁻² (ref. 1). This observation is remarkable considering that the kestrel eye is only half the size of the human eye. It has been suggested that two anatomical/optical features of the kestrel eye account for this level of performance: the reduced centre-to-centre spacing of photoreceptors² and the foveal pit which has been hypothesized to serve as an image magnifier³.

The highest cutoff frequency, ν , observed for human at high levels of illumina-

tion is 57–60 cycles deg⁻¹ (refs 4, 5) and is predicted by focal length, F (≈ 17 mm), and centre-to-centre spacing of foveal photoreceptors, d_{cc} (≈ 3 μ m)²:

$$\nu = \frac{F}{d_{cc}\sqrt{3} \times 57.3} \times m$$

However, the observed cutoff for the human observers in this study was only ≈ 40 cycles deg⁻¹ as expected due to the moderate level of illumination^{6,7}. Therefore, the limitation of human cutoff frequency due to illumination was 40/60 or ≈ 0.66 .

Kestrel acuity has been reported to fall off 2.4 times faster than human for levels of illumination below 350 cd m⁻² (ref. 8). The optimal cutoff frequency for kestrel estimated from the above equation (where $F = 9.1$ mm and $d_{cc} = 2$ μ m) is 46 cycles deg⁻¹. Making the conservative assumption that the illuminance limiting factor for the kestrel is the same as for human, the expected cutoff frequency for the kestrel in this experiment is ≈ 0.66 (46 cycles deg⁻¹) or ≈ 30 cycles deg⁻¹; using the kestrel data cited above the expected cutoff is between 28.5 and 29 cycles deg⁻¹. Hence, I propose that foveal magnification boosts the expected cutoff frequency of ≈ 30 cycles deg⁻¹ or less for the moderate level of illumination in this experiment to the observed value of 40 cycles deg⁻¹.

In addition, Dvorak, Mark and Reymond complain that the final data point for the stationary condition in my study was 25 cycles deg⁻¹ and suggest that 'perhaps 25 cycles deg⁻¹ is the appropriate cutoff frequency'. However, as the two functions (stationary and phase changing gratings) merge above 10 cycles deg⁻¹, thresholds were not determined more than once above 25 cycles deg⁻¹. Data points were simply connected with a smooth line and the final observation was at 40 cycles deg⁻¹. Therefore, the 40 cycles deg⁻¹ cutoff frequency is an observed and not an 'interpolated' value.

Their argument for no foveal magnification requires that the observed cutoff frequency of 40 cycles deg⁻¹ was only marginally luminance-limited and, therefore, that 40 cd m⁻² is a nearly optimal level of illumination for the kestrel. As pointed out above, this assumption is not consistent with previous data for either human or kestrel or with previous arguments by Reymond and Wolfe⁹ to account for the very poor visual performance observed for the eagle at an average illumination of 20 cd m⁻².

To summarize, in what is the first published avian MTF study (which characterizes performance of the complete visual system), I reported the surprising finding that the kestrel's spatial frequency cutoff is the same as that of the human for moderate photopic illuminances and pointed out that this finding may be explained by the kestrel's higher receptor packing

density and a magnification factor¹. Dvorak *et al.* contend that the magnification factor is not necessary to explain the data but their argument is only valid, all other things being equal, if the kestrel's fall-off in acuity due to low illumination is much less rapid than that of the human, which seems unlikely. The best available anatomical data of focal length and receptor density alone cannot account for the remarkable visual acuity of birds of prey. Therefore, the magnification factor remains plausible, even though its existence has yet to be confirmed experimentally for birds of prey as it has been for other species¹⁰. Thus, the proposed optical magnification function of the deep fovea³ remains the most likely explanation for the exceptional visual acuity of birds.

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Carbon geodynamic cycle

AN estimate has recently been presented of the rate of exchange of carbon dioxide between the mantle of the Earth and the atmosphere and ocean¹. Basing their argument principally on the concentrations of the stable isotopes of carbon in mid-ocean ridge basalts, Javoy *et al.* arrive at a best estimate of the flux of carbon dioxide from the mantle to the ocean equivalent to 3.9×10^{14} g C yr⁻¹. They suggest that subduction of carbonate sediments carries an equivalent flux of carbon back into the mantle.

This estimate is almost certainly too large, probably by a substantial factor. Carbon is removed from the fluid phase (ocean and atmosphere) and restored to the solid phase (sea floor sediments) principally by reaction with dissolved calcium and magnesium ions to form carbonate minerals. An estimated 13×10^{14} g Ca²⁺ yr⁻¹ or 7.8×10^{14} g Mg²⁺ yr⁻¹ would be needed to neutralize the proposed carbon dioxide flux of 3.9×10^{14} g C yr⁻¹, but rivers carry to the ocean only an estimated 5×10^{14} g Ca²⁺ yr⁻¹ and 1.4×10^{14} g Mg²⁺ yr⁻¹ (ref. 2). Much of this river-borne flux of calcium and magnesium ions is derived from the weather-

ing of carbonate minerals³, and so does not contribute to the neutralization of carbon dioxide released from the mantle. Interaction between seawater and sea floor basalts releases a maximum flux of 2×10^{14} g Ca^{2+} yr^{-1} (refs 4, 5) and leads to a net consumption of magnesium ions. So large a flux of carbon dioxide from the mantle to the ocean would therefore imply a geologically rapid accumulation of carbon dioxide in the atmosphere and ocean.

Carbon can be removed from the fluid phase and restored to the solid phase also by the incorporation of reduced organic carbon in sediments. This process is estimated to occur at a rate of 1.2×10^{14} g C yr^{-1} (refs 3, 6), but this flux must be largely cancelled by the rate of oxidation of old organic carbon in sedimentary rocks in order to conserve the oxygen content of the atmosphere.

There are $\sim 4 \times 10^{19}$ g C in the atmosphere and ocean combined³. An unbalanced flux of carbon dioxide from the mantle to the ocean of the magnitude suggested by Javoy *et al.* would therefore lead to a doubling of the carbon content of ocean and atmosphere in a time of only a few hundred thousand years.

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JAVOY *ET AL.* REPLY—We thank Walker for his comments which are very relevant to our article. If one wants to recycle carbon into the mantle through the sediments one has to find carriers which actually belong to the sediments and calcium carbonate is the most obvious, together with organic matter. These are the only recycled species that we have considered in our sedimentary carbon budget. However, we do not think that the values presented by Walker seriously put into question our evaluations for three reasons.

First, any evaluation of geochemical flux is subject to relatively large uncertainties as is the case for our estimates of the mantle carbon flux from the mid-ocean rises ($0.58\text{--}7.2 \times 10^{14}$ g yr^{-1}) and sedimentary carbon flux to the mantle ($2.8\text{--}5.6 \times 10^{14}$ g yr^{-1}). This is also true for Ca^{2+} and Mg^{2+} fluxes from continents or from ocean floor hydrothermalism for which the values given by Walker from his refs 2–5 are true within a factor of 2, but any better precision is doubtful.

However, we shall deal with the values as given and only correct our results because of a remark by Francheteau, who brought to our attention the calculations of Parsons¹: the lithospheric surface created and destroyed is $3 \text{ km}^2 \text{ yr}^{-1}$ and not $4.8 \text{ km}^2 \text{ yr}^{-1}$ as we stated. Hence our results have to be divided by 1.6, giving a mean estimate of 2.44×10^{14} g yr^{-1} for the mantle carbon flux in both directions.

Second, the most important comment to make is that we cannot distinguish between the different carbon sources in the calcium carbonate present in marine sediments: we only know that a certain flux of Ca^{2+} and Mg^{2+} is carried to the oceans from continents and hydrothermal circulations. Taking the estimates given by Walker for calcium alone (7×10^{14} g yr^{-1}) we see that this can recycle 2.1×10^{14} g yr^{-1} of carbon to the sediments. With the addition of sedimentary organic carbon in the proportions given in our paper (one-third of the total sedimentary carbon) we get 3.15×10^{14} g yr^{-1} . In a steady-state model, this recycled carbon comes from the only possible important source external to the exogenous cycle, that is, the upper mantle carbon flux (2.44×10^{14} g yr^{-1}). These two values agree within reasonable limits. The first one is greater, which, in a steady-state model, implies that mantle contributions from sources other than mid-ocean ridges is 7×10^{13} g yr^{-1} .

This type of calculation is similar to those carried out by Craig² and Wickmann³ in their evaluation of the $^{13}\text{C}/^{12}\text{C}$ ratio of mantle carbon, except that they did not calculate the fluxes or postulate recycling into the mantle.

Systematics of basidiomycetes based on 5S rRNA sequences and other data

WALKER and Doolittle¹ have recently suggested a fundamental rediagnosis of the basidiomycetes on the basis of 5S rRNA sequences. They also suggested that certain morphological characteristics “are probably of independent origins” and that there are “contradictory combinations of traits involving reproductive structures and spore germination that traditionally separate the homobasidiomycetes and heterobasidiomycetes”. I intend here to provide support for a basic division they propose, but also to show that there need be no contradictory combinations of traits or traits with independent origins in the basidiomycetes.

I have recently developed algorithms for reconstructing phylogenies from restriction site² or sequence³ data and, more important, for testing alternative phylogenetic hypotheses against one another. For sequence data, a maximum parsimony network is constructed for

Finally, it is worth pointing out that some of the carbon flux from the ridges may be incorporated directly into the mantle oceanic lithosphere, which, in our terminology, belongs to the external cycle.

We believe that continents and sea floor basalts provide enough calcium for the recycling of carbon fluxes into the mantle up to the levels we postulated in our model of the carbon geodynamic cycle. However, we thank Walker for forcing us to explain how our recycling could work.

We should like to take this opportunity to correct three other errors pointed out by Francheteau, whom we thank.

- (1) In ref. 22, read La Jolla and not Paris.
- (2) In ref. 23, read Washington and not Paris.
- (3) Uyeda and Kanamori (ref. 24) speak only of extension and compression but never of strong and weaker distension.

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each variable site assuming a particular phylogeny is true, and then the procedure is repeated for an alternative phylogeny. The difference in the number of mutational events for this particular nucleotide site under the two phylogenies is then used as a score. A similar score is generated for each variable nucleotide in the data set and the resulting scores are used in a Wilcoxon matched-pair signed-rank test.

Figure 1 shows the eight species studied by Walker and Doolittle along with some of their relevant taxonomic and morphological features. Walker and Doolittle concluded that the fundamental split in this group corresponds to the clusters identified on the basis of septal pore type, as indicated in Fig. 1. I tested the significance of this fundamental division and obtained a signed-rank sum of -46 versus the most likely alternative with a sample of 45 non-zero scores. This value is so low it is not listed in the standard tables⁴. Consequently, the rRNA sequence data strongly support the division suggested by Walker and Doolittle.

Educating Sarah

Richard Passingham

The Mind of an Ape. By David Premack and Ann James Premack.
W.W. Norton: 1983. Pp.165. \$14.95.

COMPARATIVE psychology has proved a dull science. Its efforts have been directed mainly to the rank ordering of vertebrate species according to their ability to learn. To this end a restricted number of tests have been applied, none of which greatly taxes the mind of an intelligent animal such as a chimpanzee.

The history of the subject has been enlivened by two minds. In 1917 Kohler pointed out that there was little point in seeking evidence for insight if the tests were of the sort that could be solved only by rote trial and error. And in 1949 Harlow suggested that rather than killing animals after a single experiment, it would be of interest to study them over a long time to see to what extent they benefited from a general education.

In recent times David Premack has made the subject very much more interesting. He has taken note of Harlow's suggestion and has trained a chimpanzee, Sarah, for three to four hours a day, five days a week for over ten years. Also, he has heeded Kohler's insistence that it is more important to know whether an ape understands the nature of a problem than whether it can be drilled to produce the right answer. For each type of problem Sarah is first taught the answers for a training series and then tested for her understanding on a new set of problems that are formally similar. If she can give the correct answer on the first trial of such problems she must have an intelligent understanding.

What are Sarah's achievements? This book summarizes in popular form some years of work by David Premack and his associates (Woodruff, Gillan and others), much of which has already been reported in the technical literature. In Chapter 2 they claim that Sarah can master the abstract idea of similarity, recognizing that two bananas are like two apples rather than an apple and a banana. And they report that Sarah can also draw simple analogies; thus tin opener is to can as key is to lock. Chapter 3 describes Sarah's efforts to supply the correct solution to problems that she sees people tackling on the television screen. It is argued that her ability to produce the correct solutions suggests that she understands the problems the people are facing and their goals or intentions. The next chapter looks at her understanding of her physical world; does she appreciate that events have causes or that matter can be conserved in amount from one physical transformation to another?

But there is more to this account than a simple listing of achievements. Earlier in her life, Sarah was drilled in the use of a system of visual signs. Could it be that her later successes were promoted by this previous training in a symbolic system? Not in the sense that she solved the problems by mentally operating with these symbols, since she lacked symbols for many of the essential operations. But, David Premack argues, perhaps the earlier training taught her to look for abstract relationships. And indeed there is evidence for this, since three other chimpanzees were also taught the symbolic system and four other chimpanzees, now juvenile, are being tested on the logical problems

without having had the benefit of the early training with symbols.

The reader may wish to carp, however. Has it not turned out that some of the early claims for language learning in chimpanzees were inflated? Perhaps there are alternative and rather less interesting explanations of Sarah's performance. Perhaps some of the explanations can be translated into the stultifying jargon of behaviourism.

It is worth haggling over these questions because for once there is something worth haggling about. Of course there are alternatives and Premack frequently mentions them and tries to rule them out. But this is a small book and does not give the detail that is needed to allay the sceptic's fears. *The Mind of an Ape* is nonetheless full of imaginative questions and ingenious experiments. If children have their Piaget, chimpanzees have their Premack. □

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Power and politics

John Chesshire

World Nuclear Energy: Toward a Bargain of Confidence.
Edited by Ian Smart.
Johns Hopkins University Press: 1983.
Pp.394. \$30, £22.

EISENHOWER'S "Atoms for Peace" declaration of December 1953 heralded a period of impressive and largely untroubled nuclear cooperation, offering assistance in the development of peaceful uses of atomic energy in exchange for the promise that such assistance would not be diverted for military purposes. Verification was to be undertaken through a system of safeguards enforced by an inspectorate, a principle that was enshrined in the creation of the International Atomic Energy Agency in 1959. The emerging bargain of confidence was taken a significant stage further with the Non-Proliferation Treaty of 1968.

By the early 1970s, therefore, not only was an international framework for nuclear power development evolving satisfactorily, but an increasing number of countries had embarked on sizeable nuclear power programmes and few had mastered more sensitive technologies such as enrichment and reprocessing. Thus the period between 1953 and 1973 can now be viewed as the golden era of cooperation — although, with hindsight, optimism within the nuclear community probably led to a glossing over of incipient tensions and an underestimation of the fragility of international nuclear diplomacy.

When India exploded a nuclear device in

1974 this community was shocked, perhaps more than anything at its own naive complacency. Amongst many other responses were the creation of the "London suppliers club" in 1975 and a hardening of US policy evidenced in the Nuclear Non-Proliferation Act of 1978. Further, at the same time the economic ground of nuclear power was shifting. The oil "crisis" of 1973 had led to huge increases in planned nuclear generating capacity, an unseemly scramble for uranium supplies and calls for greater expenditure to support the rapid development of fast reactor and fusion technologies. But the late 1970s witnessed reduced forecasts of economic growth and electricity demand, lengthening construction lead times, cost escalation, patchy plant operating performance and the incident at Three Mile Island. Both the diplomatic and economic bargains were in doubt.

These looming uncertainties were important factors leading to the formation of the International Consultative Group on Nuclear Energy (ICGNE) in 1977, under the co-sponsorship of the Rockefeller Foundation and the Royal Institute of International Affairs. Almost contemporaneously, the International Nuclear Fuel Cycle Evaluation (INFCE) exercise was launched to undertake an essentially technical assessment of the relationships between civil nuclear power and nuclear weapons proliferation.

In contrast to the large-scale and official intergovernmental nature of INFCE, the ICGNE group comprised some twenty individuals only, meeting privately and in their personal capacities. As such, its deliberations were far less inhibited, ranging over many of the vexed international, political and economic issues

confronting nuclear power. *World Nuclear Energy* draws together, with some minor editing and updating, the eight papers produced under ICGNE's auspices between 1978 and 1980. The book concludes with the Group's admirably lucid and concise summary statement.

The essays fall into two groups. The first, primarily concerned with dynamics of international relationships and responses, embraces the historical context of global cooperation from the perspectives of both developed and developing countries; the evolution, failures and future prospects of safeguard procedures; and the prospects for international custody of plutonium stocks. The second group focuses on the economic and technical constraints on the wider diffusion of thermal and fast reactors, covering nuclear plant construction lead times, the viability of the civil nuclear power supplies industry and alternative paths for nuclear energy development to 2020 (including an assessment of cumulative uranium requirements and the implications for the deployment of fast reactors).

Whilst the book clearly identifies the risk that earlier, relatively unconstrained, forecasts of nuclear plant requirements might prove to be hopelessly optimistic, even its own, more sober, estimates have been overtaken by events. Although the Group, rightly in my view, calls for fuller and more effective cooperation in fast reactor development, this is rapidly emerging as the conventional wisdom — even in France, given the difficulty that country now faces in justifying independent attempts at commercialization.

The Group's other recommendations have more fully stood the test of time. The authors argue the urgent need to demonstrate the commercial feasibility of large-scale reprocessing of spent fuel from thermal and fast reactors, and for international attention to be given to nuclear waste management. They consider that the main impediment to international collaboration and trade is the fear of weapons proliferation; and that whilst existing nuclear arsenals are in fact the greater risk to international security, it is fear of further proliferation which hampers nuclear relations between states.

Hence the need for a new bargain of confidence. The key elements of such a bargain include credible assurances of the continuity of international enrichment and reprocessing services (to reduce pressures for further national development of such facilities), and the conduct of such operations under multinational auspices as well as technical safeguards. The essays in this book comprise an invaluable assessment of these and other issues which are likely to be on the nuclear agenda for the next two decades or more. □

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Thinking about food webs

Michael Gilpin

Food Webs. By Stuart L. Pimm.
Chapman & Hall: 1982. Pp.219. Hbk £15, \$35; pbk £7.50, \$16.95.

THE title of this important and long-needed book is misleading. It sounds like a successor to the seminal work by Joel Cohen, *Food Webs and Niche Space* (Princeton University Press, 1978). Actually, it advances, both with widely collected empirical data and with new, more realistic models, the stability-diversity controversy, which has not been adequately reviewed since Robert May's *Stability and Complexity in Model Ecosystems* (Princeton University Press, 1975).

Most of the theoretical models discussed by May were of the random coefficient type. Work since then, much of it contributed by Pimm and his co-investigator, John Lawton, has added some *a priori* structure — a web pattern — which constrains the signs and magnitudes of the otherwise random coefficients. This complicates the analysis, but it has very much enriched the conclusions.

Pimm begins with a long review of the pertinent stability mathematics, which he warns, I believe correctly, will offend nearly everyone because it is at the same time both dull and daunting. A particular problem is his insistence on Lotka-Volterra, for this precludes investigations

of the robustness so sorely needed. And a possibly more important weakness is his almost total attention to stability at the expense of discussion of the feasibility (coexistence) of his species assemblages.

The second half of the book treats a series of somewhat unrelated problems. Most interesting, I believe, is Pimm's analysis of the constraints on the length of food chains. The textbooks tell us this is simple thermodynamics: by the "rule of 10" only a tenth of the energy in any one trophic level is passed to the next. Thus, by the fourth or fifth level, there is not a sufficient flow of energy to support such carnivores. Pimm marshals data to refute this. And then he presents his own ideas, which are based on the instability and extended times for return to equilibrium of such long dynamic chains, thus making the highest feeders the whip end of normal environmental noise.

A further chapter questions some of the statistical methodology Cohen used to support his conclusions regarding the unidimensionality of food webs. This parallels other recent criticisms and reappraisals.

The final chapter sorts out the various thrusts of Pimm's investigations and summarizes them in excellent graphical form. The pattern of these conclusions must be considered to reflect the current status of thinking on food webs. As such, Pimm's book should attract a wide audience and should serve, for years to come, as the basis for new investigations, both theoretical and empirical. □

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What's so funny about computers? A Sidney Harris cartoon, taken from his collection of that title recently published by William Kaufmann. Price of the book is \$6.95, £5.80.

"As I see it, it's a toss-up between a Belgian data processing machine and an American electronic computer."

Pollution from the Soviet Union

A.J. Southward

Pollution and the Biological Resources of the Oceans.

By S. A. Patin.

*Butterworth Scientific: 1983. Pp.289.
£35, \$89.95.*

THE industrial nations vary in the care they devote to protection and restoration of the marine environment, and a sort of pecking order has been informally recognized. Soviet Russia has generally been placed rather low in this ranking system, and the popular scientific press has carried stories about the destruction of natural communities in the Caspian Sea and Lake Baikal. It is therefore a pleasant surprise to encounter this updated translation of Dr Patin's book, which gives an account of Soviet investigations of marine pollution as part of a general review of the subject.

Dr Patin is a radiochemist who later moved into the field of experimental ecotoxicology of fish, and these two aspects are well-covered in the book. The introductory chapter describes the general background to pollution studies, and gives definitions of terms and concepts. It is worth noting the author's implied criticism of autecological investigations — concentration on one organism to the exclusion of the rest of the ecosystem — and of the dilution concept so often used to excuse discharges into the sea. Dr Patin states firmly that the toxic criteria for sea water should include changes in production and species composition, disappearance of sensitive species, alterations to biochemical pathways and disturbances to the ecological balance; he is clearly not one of those persons who regard the presence of any form of life, however restricted in diversity, as an indication that pollution is not harmful.

The next chapter describes patterns of world pollution, and an attempt is made to formulate a global model of epipelagic pollution. Attention is drawn to localization of pollution adjacent to industrial sources and to the concentration of many pollutants in a thin layer at the air-water interface. Chapter 3 tabulates occurrences of pollutants such as radiochemicals, oil, heavy metals and chlorinated hydrocarbons. The author then shows how these substances are distributed and accumulated in marine organisms, using both graphs and tables. His summary of the incidence of heavy metals and chlorinated hydrocarbons in commercial marine products is especially useful, in that it is accompanied by statistical parameters that show the degree of individual variation. It is made quite clear that heavy metal content increases in the general order oceanic-neritic-inland sea; fish from the Caspian

contain nearly twice as much mercury (0.275 mg/kg) in their muscles as do fish from the North and Barents Seas, which in turn contain twice as much as fish from the Atlantic Ocean.

Dr Patin then turns to methods of investigation — including a lucid explanation of toxic effect areas and permissible (reversible action) zones in three-dimensional plots — and reviews the experimental approach with unicellular algae in the laboratory and the field; he recommends monitoring changes in rate of photosynthesis as a more sensitive indicator than cell division.

The section on animals is arranged in systematic order, and among fishes it is interesting to see how sensitive to pollution are the eggs of sturgeon and some local forms of salmonids compared with typical test species such as cyprinids. Following two chapters summarizing general features in distribution of pollution and the

biological consequences, the final chapter offers suggestions for control and monitoring. The author ends by stressing that the scientific and technological advances that have caused pollution could equally be employed in reducing the volumes of harmful substances emitted. He admits, however, that the search for such solutions and their full-scale realization will be expensive, and that there is little prospect of substantial reduction in marine pollution in the next decades.

This is a readable book, well-translated and produced apart from a few misprints and peculiar Russianizations of the names of Western researchers. Not only does it provide a good introduction to the whole subject of marine pollution, but also many valuable insights into otherwise inaccessible Soviet researches. □

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Light on experiment

P.M. Rentzepis

Experimental Methods in Photochemistry and Photophysics.

By J.F. Rabek.

*Wiley: 1982. 2 volumes, pp.1,098.
£89.95, \$189.50.*

THE SUBTITLE of these two books might well have been "An Encyclopaedia of Experimental Photochemistry"! Starting with the "Nature and Properties of Light" and "Optical Radiation Sources", Rabek proceeds to discuss in simple, coherent language the basic aspects and the instruments used in photochemistry and photobiology. From sunlight to picosecond lasers, Rabek makes a successful attempt to provide the photochemist with a working knowledge of instrument components, their designs and their modes of operation. Rather than engulfing the reader in theory and mathematics, the author describes in a straightforward fashion how and under what conditions each component operates efficiently in the laboratory.

Chapter 4, which covers in about 70 pages the various lenses and prisms most commonly used by the photochemist, provides the bare minimum of background on the theoretical principles of optics. Such material is found in other texts but was usefully included here to inform the photochemist or photobiologist of component composition, characteristics and applications.

The way in which Rabek discusses and illustrates the various optical components reminds one, in some respects, of the descriptions to be found in manufacturers' catalogues. The accounts are nonetheless generally informative and provide the necessary insight for the construction of experiments, although in places the treat-

ment is very elementary and will be of use only to neophytes.

The chapters on photodetectors, especially the one dealing with multi-channel detectors, are especially timely and well written. They provide the essential information for the selection and operation of vidicons and optical multichannel analysers, instruments which are becoming essential in many spectroscopic experiments. Similarly, the chapter on signal recovery includes a list of the instruments available, for example oscilloscopes, lock-in amplifiers, photon counters and optical multichannel analysers.

The second volume is mostly concerned with laser and fast spectroscopic measurement techniques, although there is a short description of electronic transitions which gives only definitions of terms. The chapter on emission spectroscopy is written for newcomers to the field rather than specialists, as indeed are all of the basic chapters in both books. There is, however, an extensive account of actinometry which is broad in scope and examines most of the actinometric and radiometric methods used by photochemists interested in absolute quantum yields. The treatise ends with a pertinent description of the more common hazards in photochemical research and the means for avoiding them.

These two books are well written, easily understood and encompass most of the necessary information for the practice of photochemical research. They are not texts on the theoretical principles of the subject, but rather an encyclopaedic compilation of instruments for photochemists and photobiologists. As such they provide a convenient and easily accessible source of information which is difficult to find elsewhere. □

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Africa: war against health

Len Goodwin

'Bid the Sickness Cease': Disease in the History of Black Africa.

By Oliver Ransford.

John Murray: 1983. Pp.235. £12.50.

*Take up the white man's burden,
The savage wars of peace.
Fill the mouths of famine
And bid the sickness cease.*

Kipling has gone out of fashion and, as the author of this book says, his most maligned poem may sound ridiculously quaint and hypocritical nowadays. Nevertheless, there was far more than arrogance and greed in the European Powers' attitude to the African peoples after the intrusions of the 1880s. There was also a growing philanthropic impulse to improve the standards of living and health of faraway subjects, and many men and women volunteered to serve in the administration or the missions although they knew that, for many of them, a posting to Africa was a sentence of death.

Dr Ransford himself joined the Colonial Medical Service in 1938 and served first in Nyasaland (Malawi) and later in Rhodesia (Zimbabwe) where he still lives. In *'Bid the Sickness Cease'* he traces the history of Black Africa and the effects upon its peoples of the spread of disease, both endemic and epidemic, brought about by migrations that followed tribal wars, the slave trade and, most profoundly, the opening up of the continent by Europeans when protection from malaria by quinine enabled them to survive journeys into the interior.

Spread of originally localized indigenous infections such as trypanosomiasis, and murderous epidemics of smallpox, plague, measles and influenza made the years between 1885 and 1930 the most unhealthy period of African history — in one area of French West Africa the population decreased from 20 million in 1911 to 2.5 million in 1931. In this respect, however, Africa did better than the Amerindians or the Pacific aborigines, whose populations never recovered from the savagery of introduced epidemics.

The author deals with the principal communicable infections of Africa — malaria, sleeping sickness, yellow fever and bilharzia —, lesser terrors such as malnutrition, leprosy, yaws, dysentery and filariasis, and epidemics of plague, cholera, influenza, cerebrospinal fever and smallpox. He outlines, *en passant*, the natural history of the parasites responsible, and the biographies of the major personalities associated with their discovery. These are sensitively and often humourously done; he even evokes some sympathy

for the impossible, obsessional Ross and the arrogant Bruce, and he gives a scrupulously fair account of the first-class row that developed with Castellani over the discovery of the trypanosome as the cause of sleeping sickness. The structure of the argument makes the book interesting and easy to read, and scattered snippets of recondite information give it colour and perspective. Ransford's deep, but not slushy, understanding and feeling for his patients and their problems runs throughout. He has read and consulted widely and there are few errors — mostly quibbles raised by the simplification of complex or debatable material.

But what of the future? Dr Ransford has confidence in the efforts of the World Health Organization to bring about "Health for all by the year 2000", although he warns that time is short and that WHO had better hurry up. More effective medicines and vaccines are urgently needed, and the pharmaceutical industry is not yet sufficiently interested in producing them — the research is difficult, expensive and chancy, new discoveries are pirated by others who have spent nothing on research and development, and the people who need the drugs are too poor to pay for them anyway. Unless measures are taken soon by

the Northern countries to improve the standards of living and the economies of the South — as emphasized in the Brandt Report — crisis is imminent for the North as well as the South.

But the major obstacle to health in Africa is political instability. As Ransford says, with the coming of independence to the ex-colonial territories, Africa has now taken its place in world affairs and has begun to move into a future of its own making; but great vision is needed, and development can only take place in a continent at peace within itself. This, at present, is not a reality. William McNeill in his book *Plagues and Peoples* (on which Ransford draws for his historical perspectives) has shown how diseases spread by the movements of armies and refugees and the destruction of agricultural communities by war have profoundly affected the course of history. All this is still going on in Africa and there seems to be little hope for health by the year 2000 unless someone can bid the fighting cease. □

Len Goodwin was formerly at the Wellcome Laboratories of Tropical Medicine and later Director of Science at the Zoological Society of London. Now retired, he is a part-time consultant to the World Health Organization.

Simply gauge theory

John Ellis

An Informal Introduction to Gauge Field Theories.

By I.J.R. Aitchison.

Cambridge University Press: 1982.

Pp.171. £12.50, \$22.50.

IAN Aitchison has previously written with A.J.G. Hey a useful introductory text on gauge theories, suitable for experimental students. The present volume aspires to fill a gap between that earlier book and the more advanced treatises such as that by Itzykson and Zuber. It is intended for novices to the subject such as graduate students and outsiders (cosmologists perhaps?) who might want to learn about gauge theories because of their significance for their own field.

Aitchison is an experienced and greatly appreciated teacher who has often taught graduate courses and lectured at summer schools. His selection of material and manner of presentation is undoubtedly influenced by these experiences, and responds to a genuine need. Nonetheless, although I find the concept of the book appealing, and much of the material well selected, I do have some reservations about the way it is presented.

Aitchison starts with a somewhat sparse chapter on the motivations for studying gauge theories, and then comes back to their applications at various points in the text. I found this approach a little un-

settling, and it would perhaps have been better either to drop the accounts of motivations and applications entirely, or else to discuss them more coherently and completely. The author then introduces symmetry in quantum field theory in some detail, a topic which would not appear to present great difficulties. I know from experience, however, that students often appreciate such a systematic approach.

The sections on gauge theories and their quantization do not employ the path integral formalism. It is perhaps a matter of taste whether this omission is sensible nowadays, but it may be appropriate at this introductory level. The book concludes with a section on renormalization, but this is incomplete in that there is no discussion of the renormalization of non-Abelian gauge theories. Why not calculate the β -function?

The book is also marred by some nagging slips. The Noether theorem is only called by its name several pages after its proof. The punctuation is sometimes baroque — see an egregious sentence on p.30, for example. Some distinguished physicists' names are consistently misspelt. And the mass of the charm quark was originally estimated from $K_s K_L$ mixing, not from $K_L^0 \rightarrow \mu^+ \mu^-$ as asserted on p.119.

Despite these minor defects, newcomers to the subject may find the book useful, though I think most theoretical students will prefer to consult more advanced texts. □

John Ellis is at the Stanford Linear Accelerator Center, Stanford, California.

Awards

Applications are invited for the 1983 André Lichtwitz prize for outstanding clinical or fundamental research on calcium and phosphorus metabolism. The prize of 11,000 francs is awarded annually by the French INSERM (Institut National de la Santé et de la Recherche Médicale) to either an individual or to a team conducting research in this field. Conditions of entry for the 1983 prize may be obtained from INSERM (c/o Mlle C. Chirol, 101 rue de Tolbiac, 75654 Paris Cedex 13, France) and applications should reach the director of INSERM by 30 September 1983.

The Ciba Foundation has established a bursary scheme to enable young scientists to attend Ciba Foundation symposia and to subsequently spend one month in a symposium participant's laboratory. Applications are sought from scientists aged 25–35 who are actively engaged in research in the following fields, all of which will be the subject of symposia in the first half of 1984: basement membranes, mucous and mucosa, the value of preventive medicine, biological transformations in organic chemical synthesis, and microbial toxins. The bursary will provide for travel costs and board and lodging for the period of the bursary. Applications, together with a full curriculum vitae, details of the candidate's current research, a statement of the candidate's career aims and the names of two referees, should be addressed to The Director, Ciba Foundation, 41 Portland Place, London W1N 4BN, UK. They should be received by 30 September 1983.

Applications are invited for the British Medical Research Council's non-clinical senior research fellowships. Applicants should be resident in the UK, untenured, but with not less than three year's post-doctoral experience by the date on which they would take up their fellowship. They should be between 26 and 32 years of age on 30 September 1983. The fellowships provide personal support for five years, with provision for supporting staff, expenses and equipment for the first three years. After two and a half years each fellow would be invited to submit a formal application for the continuation or expansion of the provision for supporting staff and expenses over the remaining period of the award. Fellows may apply for an extension of the fellowship for a second five year period on the same basis. Application forms may be obtained from the Grants Section, Medical Research Council, 20 Park Crescent, London W1N 4AL, UK. The closing date for entries is 30 September 1983.

The Linnean Society of London has

presented awards to the following people: Linnean Medals were presented to **Professor M.J.D. White** for his contributions to cytogenetics and evolution and **Professor C.T. Ingold** for his work on fungi and his services to biological education in Britain and overseas. The Bicentenary Medal for a biologist under 40 has been awarded to **Dr J.R. Krebs**, for his analyses of the ecological and evolutionary significance of behaviour, and the H.H. Bloomer Award for contributions by an amateur has been given to **Mr O. Polunin** for his works on botanical guides and his studies of the flora of the Himalayas. In addition, two foreign members have been elected to the society — **Professor Z.K. Jaworowska** from Poland and **Dr Wu Hsien Wen** of the People's Republic of China.

The Lister Institute of Preventive Medicine has awarded research fellowships to the following: **Dr R. Beddington**, **Dr L. Borysiewicz**, **Dr J. Fry**, **Dr B. Halliwell** and **Dr C. Higgins**. The fellowships are tenable for five years. The Lister Institute fund for fellowships for research in biomedicine was set up after the Lister Institute laboratories at Elstree in Hertfordshire, UK, were disbanded.

Applications are invited for the British Medical Association film awards. The gold, silver and bronze awards are given to educational films of outstanding merit and are designed to encourage the production of material which is more effective in medical education. All films entered for the competition will be automatically considered for the BLAT Trophy, given to films of high educational standard, and the Harold E. Lewis Award, which was established in 1975 by the Board of Science and Education of the British Medical Association to commemorate the work of the late Dr Harold Lewis. Further information and entry forms may be obtained from the Film Librarian, The British Medical Association, BMA House, Tavistock Square, London WC1H 9JP, UK.

Miscellaneous

A brochure containing information on international courses, fellowships and scholarships in hydrology has been produced by UNESCO. The first part of the brochure describes the international post-graduate courses that are sponsored by UNESCO within the International Hydrological Programme. The second part lists the scholarships and fellowships that are awarded by several countries to foreign students as part of a national teaching and development programme. The third part gives detailed information on special conditions governing the award of fellowships, and also gives details of course curriculae

and additional national training facilities.

Appointments

Dr Roman Kintanar, director general of the Philippine Atmospheric, Geophysical and Astronomical Services Administration has been re-elected as president of the World Meteorological Organization (WMO). The WMO Congress has also appointed **Dr Godwin Olu Patrick Obasi**, the current director of the WMO's education and training department, as secretary general of WMO.

Professor D.C. Smith, Sibthorpe professor of rural economy at the University of Oxford, has been elected biological secretary of the Royal Society with effect from 1 May 1983. He succeeds Sir David Phillips, who has resigned after being appointed chairman of the Advisory Board for the Research Councils.

Sir Monty Finniston has been appointed chairman of the council of Chelsea College, University of London. He succeeds Dr Miles Weatherall, who has resigned his post after 13 years. Sir Monty takes over the chairmanship at a time when the college is involved in a tripartite merger with King's College and Queen Elizabeth College, both in the University of London. He will play an important part in the future negotiations, being part of a six man team made up of the three college principles and the three chairmen of the college councils. Sir Monty brings to the college an interesting combination of academic excellence (he was vice president of the Royal Society from 1971 to 1972) and business acumen. He is currently either chairman or director of ten British companies, and president of six national institutions, including the Association of British Chambers of Commerce and the Industrial Building Bureau. His qualifications not only make him highly suited to his part in the merger talks, but, in times when finances are limited, his connections with high-technology business could be a useful asset to a college already specializing in science and engineering.

The British National Computing Centre has appointed **Professor John Ashworth**, the vice chancellor Salford University, as its chairman. The Centre was set up in 1966 as a government-aided information bank, affiliated to the Department of Industry. It is now an independent concern with a turnover of around £8 million, although it performs essentially the same functions as before and still retains some advisory responsibilities to the government. As chairman, Professor Ashworth will be involved both with the running of the company and will also represent the government's interests within the Centre.

Meetings

5-7 August, **Allergy, Immunology and Rheumatology**, La Jolla, California (Ms D. Tissue, Department of Academic Affairs, Box 400S, Scripps Clinic and Research Foundation, 10,666 North Torrey Pines Road, La Jolla CA 92037 USA).

24-26 August, **Annual Symposium of the Uranium Institute**, London (Miss R.M. Stanger, Symposium Secretariat, Conference Associates UIS, 34 Stanford Road, London W8 5PZ, UK).

7-9 September, **Lactic Acid Bacteria in Foods: Genetics, Metabolism and Applications**, Wageningen, The Netherlands (Dr P.M. Klapwijk, Symposium Secretary, c/o Unilever Research Laboratorium Vlaardingen, PO Box 114, 3130 AC Vlaardingen, The Netherlands).

11-16 September, **Harden Conference on the Molecular Basis of Virulence in Bacteria and Certain Parasites**, Ashford, UK (Executive Secretary, SGM, Harvest House, 62 London Road, Reading RG1 5AS, UK).

18-23 September, **World Congress of Food Science and Technology**, Dublin (Dr R.L. Joseph, 44 Northumberland Road, Dublin 4, Ireland).

18-24 September, **Course on Microwave Measurements**, University of Kent (Ms C. Dagnall, Press Office, IEE, Savoy Place, London WC2 0BL, UK).

19-23 September, **International Conference on Physical Chemistry of the Solid State Applications to Metals and their Compounds**, Paris (Société de Chimie Physique, 37th International Meeting, 10 rue Vauquelin, 75231 Paris Cedex 05, France).

19-23 September, **Workshop on Training and Employment of Environmental Biologists**, London (European Communities Biologists Association, Institute of Biology, 20 Queensbury Place, London SW7 2DZ, UK).

20-22 September, **European Conference and Exhibition of Sensors and their Applications**, Manchester (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

20-23 September, **International Pneumococcal Conference**, Bochum, FRG (Professor Wolfgang Ulmer, Silikose Forschungsinstitut, Hunschedtstrasse 12, D-4630 Bochum 1, FRG).

21-24 September, **International Chromosome Conference**, Lübeck, FRG (8th International Chromosome Conference, Secretariat, Institut für Pathologie, Medizinische Hochschule Lübeck, Ratzeburger Allee 160, D-2400, Lübeck, FRG).

26-28 September, **Symposium on Turbulence**, University of Missouri-Rolla, USA (Professor G.K. Patterson, Chemical Engineering Department, University of Missouri-Rolla, Rolla, Missouri 65401, USA).

27-30 September, **International Colloquium on Physical Chemistry of Colloids**

and Interfaces: Biotechnologies and Drug Research, Paris (Dr C. Troyanowsky, Honorary Secretary of the Société de Chimie Physique, Biotechnologies/Drug Conference, 10 rue Vauquelin F 75231 Paris Cedex 05, France).

2-4 October, **Symposium of the Whitehead Institute for Biomedical Research: Forces Moulding the Genome**, Massachusetts Institute of Technology, USA (The Special Events Office, MIT, Room 7-111, Cambridge, Massachusetts 02139, USA).

2-5 October, **International Congress of Dermatologic Surgery**, Granada, Spain (Dr Vicente Delgado, General Secretary, International Society for Dermatologic Surgery and Department of Medical-Surgical Dermatology, University of Granada, OTECSA — Recogidas, 21-1, Granada, Spain).

3-4 October, **Annual Meeting of the European Working Group for Cystic Fibrosis**, Athens (Mr R. Tucker, Executive Director, Cystic Fibrosis Research Trust, Alexandra House, 5 Blyth Road, Bromley, Kent BR1 3RS, UK).

3-5 October, **International Symposium on Novel Approaches and Drugs for Obesity**, New York (Dr A. Sullivan, Director, Pharmacology Department, Hoffman-La Roche Inc., 340 Kingsland Street, Nutley, New Jersey 07110, USA).

3-5 October, **International Seminar on Indoor Exposure to Natural Radiation and Related Risk Assessment**, Capri (Dr J. Sinnaeve (DG XII-F-1), Rue de la Loi, 200, B-1049 Brussels, Belgium).

3-7 October, **Vidcom '83: the International Videocommunications Conference**, Cannes (Commisariat Général, 179 Avenue Victor-Hugo, 75116 Paris, France).

4-7 October, **International Symposium on Environmental and Thermal Control Systems for Space Vehicles**, Toulouse, France (Centre National d'Etudes Spatiales, Département des Affaires Universitaires, 18 avenue Edouard-Belin, 31055 Toulouse, France).

6-8 October, **International Symposium on Central and Peripheral Endorphins: Basic and Clinical Aspects**, Viareggio, Italy (Professor E.E. Muller, Department of Pharmacology of the University of Milan, Via Vanvitelli 32-20129 Milano, Italy).

7 October, **Symposium on Recent Developments in Neurotoxicology**, Erasmus University Rotterdam (Professor M. de Vlieger, Rotterdam University Hospital, "Dijkzigt", Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands).

8-15 October, **World Wilderness Congress**, Scotland (The Secretariat, Third World Wilderness Congress, The Park, Forres, Scotland IV36 0TZ, UK).

10-12 October, **International Meeting on Transportation and Communications: Telecommunications and Navigation Aids in Transportation**, Genoa (Segreteria Generale dell'Istituto Internazionale delle Comunicazioni, Villa Piaggio, Via Per-

tinace, I-16125 Genoa, Italy).

12-14 October, **Analyticon '83 and Laboratory '83**, London (Mr G.C. Young, SIMA, Leicester House, 8 Leicester Street, London WC2H 7BN, UK).

16-20 October, **International Conference on Basement Tectonics**, Cairo (Dr J. Gallagher Jr, Vice Chairman, Conferences, Cities Service Company, PO Box 3908, Tulsa, Oklahoma 74102, USA).

17-20 October, **Annual Meeting of the Division for Planetary Sciences of the American Astronomical Society**, Cornell University, USA (Mr S.J. Ostro, Space Sciences Building, Cornell University, Ithaca, NY 14853, USA).

25-28 October, **European Conference on Prototyping**, Brussels (Mr D. Law, The National Computing Centre Limited, Oxford Road, Manchester M1 7ED, UK).

27-28 October, **International Symposium on the Human-Pet Relationship**, Vienna (Dr P. Messent, Honorary Secretary, Society for Companion Animal Studies, The Animal Studies Centre, Freeby Lane, Waltham on the Wolds, Melton Mowbray, Leicestershire LE14 4RT, UK).

31 October-2 November, **International Symposium on Salmon and Trout Reproduction**, Seattle (The Washington Sea Grant Programme, Communication Services, 3716 Brooklyn Avenue NE, Seattle, WA 98105, USA).

7-8 November, **Underwater Mining Institute**, Madison, USA (Mr G. Woock, Sea Grant Advisory Services, University of Wisconsin, 1,800 University Avenue, Madison, Wisconsin 53705, USA).

9-11 November, **International Conference on the State of the Art of Filtration**, Williamsburg (Ms M. Duck, Pressaids Limited, Bridge House, 181 Queen Victoria Street, London EC4 4DN, UK).

14-16 November, **International Symposium on HPLC of Proteins, Peptides and Polynucleotides**, Monte Carlo (Symposium Manager, Ms S. Schlesinger, 400 East Randolph, Chicago, Illinois 60601, USA).

20-25 November, **International Congress on Plant Protection**, Brighton, UK (R.T.R. Pierce, The Chiltern Agency, Chiltern View Farmhouse, Kingston Stort, Nr Chinnor, Oxfordshire OX9 4NL, UK).

27 November-2 December, **International Conference on the Biology of Marine Mammals**, Boston (Mr J.H. Prescott, Executive Director, New England Aquarium, Central Wharf, Boston MA 02110, USA).

5-8 December, **International Conference on the Role of Government in Mineral Resources Development**, Bangkok (The Secretary, The Institution of Mining and Metallurgy, 44 Portland Place, London W1N 4BR, UK).

7-10 December, **ChemAsia 83, the Asian International Chemical and Process Engineering and Contracting Show and Conference**, Singapore (ChemAsia 83 Conference, 11 Dhoby Ghaut No. 13-01 Cathay Building, Singapore 0922).